Streptogramins and a process for preparing streptogramins by mutasynthesis

The present invention relates principally to novel compounds which are related to the group B streptogramins, and to a process for preparing streptogramins by mutasynthesis. It also relates to novel genes which are involved in the biosynthesis of precursors of the group B streptogramins, and to their uses.

The streptogramins form a homogeneous group of antibiotics consisting of an association of two types of chemically different molecules; on the one hand polyunsaturated macrolactones (group A components) and, on the other hand, depsipeptides (group B components). This group comprises numerous antibiotics which are known under different names according to their origin and includes pristinamycins, mikamycins and virginiamycins (Cocito 1979, 1983).

The A and B components have a synergistic antibacterial activity which can amount to 100 times that of the separate components and which, contrary to that of each component, is bactericidal (Cocito 1979). This activity is more particularly effective against Gra positive bacteria such as Staphylococci and Streptococci (Cocito 1979, Videau 1982). Components A and B inhibit protein synthesis by binding to the 50S

subunit of the ribosome (Cocito 1979; Di Giambattista et al., 1989).

While knowledge of the routes by which each of the components is biosynthesized still remains partial to date, earlier studies, presented in Patent Application PCT/FR93/0923, have made it possible to identify several proteins, and the corresponding structural genes, which are involved in the biosynthesis of the two types of component.

Two parts can be distinguished in the process for biosynthesizing group B streptogramins:

1) Biosynthesis of the precursors, or their analogues, of the macrocycle: 3-hydropicolinic acid, L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine, L-pipecolic acid and L-phenylglycine.

2) Formation of the macrocycle from the precursors listed above, from L-threonine and from L-proline, or their analogues, with (a) possible subsequent modification(s) of the peptide N-methylation, epimerisation, hydroxylation and oxidation type.

Patent Application PCT/FR93/0923 relates, in particular, to the enzymes which catalyse incorporation of the precursors into the peptide chain of B streptogramins in the process of elongation, and also to their structural genes. These results have demonstrated the non-ribosomal peptide synthesis

character of the type B components.

The present invention relates, more particularly, to novel compounds which are related to group B streptogramins and, more precisely, to novel compounds of the pristinamycin I family (Figures 1 and 2), termed PI below, or of the virginiamycin S family (Figure 3).

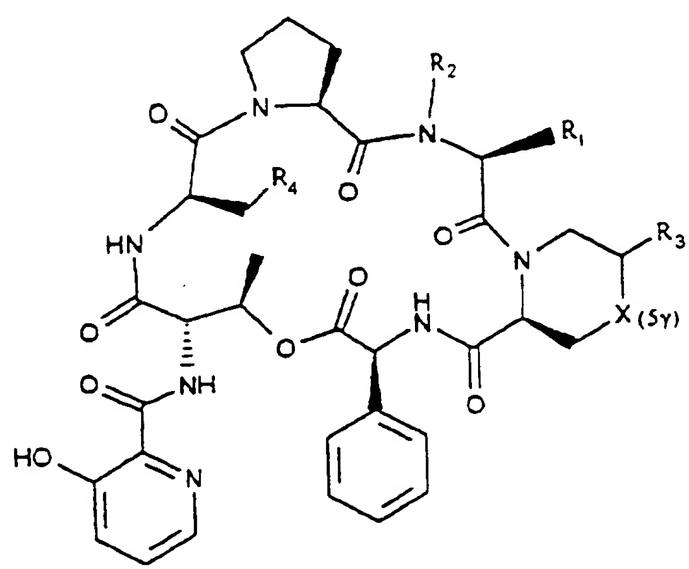
The major constituent of the I pristinamycins (PI) is PI_A (Figure 1), which represents approximately 94% of the PI, with the remaining approximately 6% being represented by minor constituents of the depsipeptide (PI_B to PI_I) whose structures are depicted in Figure 2. PI results essentially from the condensation of amino acids, certain of which are essential for protein synthesis (threonine and proline) and others of which are novel and themselves considered to be secondary metabolites (L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid and L-phenylglycine for PI_A), and also of an aromatic precursor, 3-hydroxypicolinic acid.

The virginiamycin S derivatives result from condensation of the same acids as in the case of PI, apart from 4-DMPAPA, which is replaced by a phenylalanine (see Figure 3).

Production of these different compounds by biosynthesis therefore requires preliminary synthesis, by a producer strain, of the novel precursors identified above.

The present invention results specifically from a novel process for preparing streptogramins which employs, as a strain for producing streptogramins, a microorganism strain which is mutated so as to alter the biosynthesis of the precursors of the group B streptogramins. According to this process, the said mutant strain is cultured in a medium which is supplemented with a novel precursor which is different from the precursor whose biosynthesis is altered. Unexpectedly, this results in the production of novel compounds which are related to the group B streptogramins and which are of value in the therapeutic sphere.

More precisely, the present invention relates to novel compounds which are represented by the general formula I:



I

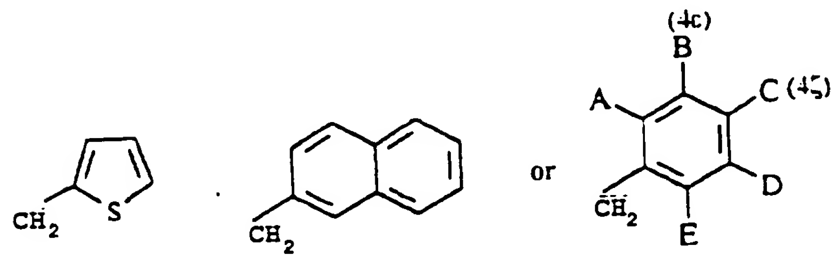
in which:

- R_2 and R_4 repr sent, independently of each other, a hydrogen atom or a methyl group,

- R_3 represents a hydrogen atom or a hydroxyl group,

- X represents a CO, CHOH or CH_2 group, and

- R_1 represents:



with

- <u>for the meta derivatives:</u>

A, C, D and E representing a hydrogen atom, and B being able to represent

- a halogen, and preferably a fluorine atom,

- a monoalkylamino or dialkylamino group, with alkyl preferably representing a methyl or ethyl group,

- an ether group; more particularly an OR group with R being preferably selected from among the methyl, ethyl, trifluoromethyl and allyl groups,

- a thioether group, preferably represented by an alkylthio group with alkyl preferably representing a methyl group,

- a C_1 to C_3 alkyl group, or

- a trihalogenom thyl group, preferably trifluoromethyl

- <u>for the para derivatives</u>

A, B, D and E representing a hydrogen atom, and C being able to represent:

- a halogen,
- an NR_1R_2 group with R_1 and R_2 representing, independently of each other, a group selected from among
  - hydrogen,
  - a straight-chain or branched C_1 to C_4 alkyl group where, when one of the substituents R_1 or R_2 represents a methyl group, the other necessarily represents an ethyl group,
  - an alkyl-cycloalkylmethyl group with a C_3 to C_4 cycloalkyl,
  - an optionally substituted C_3 to C_4 cycloalkyl group,
  - a straight-chain or branched C_1 to C_4 alkenyl group where, when one of the substituents R_1 or R_2 represents an alkenyl group, the other is different from a methyl group or a C3 to C6 cycloalkyl,
- a substituted or unsubstituted N-pyrrolidinyl group,
- an ether group; preferably an OR group with R preferably being selected from among the methyl and ethyl groups, where appropriate substituted by a chlorine atom, or trifluoromethyl and alkenyl groups
- a thioether group, preferably represent d by an alkylthio group with alkyl pref rably

representing a C_1 to C_3 alkyl group,

- an acyl or alkoxycarbonyl group and, more particularly, a COR group with R preferably representing a C_1 to C_3 alkyl group or a C_1 to C_3 alkoxy group,

- a C_1 to C_6 alkyl group which is straight-chain or branched and which is preferably selected from among the methyl, isopropyl and tert-butyl groups,

- an alkylthiomethyl group and, more preferably, a CH2SR group with R preferably representing a C_1 to C_3 alkyl group,

- an aryl group, preferably a phenyl group,

or

- a trihalogenomethyl group, preferably trifluoromethyl

- <u>for the meta-para disubstituted derivatives:</u>

A, D and E representing a hydrogen atom, and

B being able to represent:

- a halogen, preferably a fluorine atom,

- a monoalkylamino or dialkylamino group with alkyl preferably representing a methyl or ethyl group,

- an ether group and preferably an OR group with R preferably selected from among the methyl, ethyl and trifluoromethyl groups,

- a thioether group and preferably alkylthio with alkyl preferably r presenting an ethyl group, or

- a C_1 to C_3 alkyl group, and

C b ing able to represent:

- a halogen, preferably a fluorine atom,

- an amino, monoalkylamino or dialkylamino group with alkyl preferably representing a methyl group with the proviso that B is different from a bromine or chlorine atom, or a substituted or unsubstituted allyl group,

- an ether group and preferably an OR group with R preferably selected from among the methyl, ethyl and trifluoromethyl groups,

- a thioether group and preferably an alkylthio group with alkyl preferably representing a methyl group,

- a C_1 to C_6 alkyl group, or

- a trihalogenomethyl group, preferably trifluoromethyl, and

- <u>for the ortho-para disubstituted derivatives:</u> B, E and D representing a hydrogen atom and A and C a methyl group.

The following may be more particularly mentioned as preferred compounds:

4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H,

5γ-hydroxy-4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-methyl-d (4ζ-dimethylamino)pristinamycin I_H,

4ζ-methoxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-methoxycarbonyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-chloro-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-trifluoromethyl-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-trifluoromethyl-de(4ζ-dimethylamino)-pristinamycin I_H,

4ζ-tert-butyl-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-isopropyl-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-isopropyl-de(4ζ-dimethylamino)-pristinamycin I_E,

4ε-methylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ε-methoxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ε-methoxy-de(4ζ-dimethylamino)pristinamycin I_H,

4ε-fluoro 4ζ-methyl-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-diethylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-allylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-diallylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-allylethylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-ethylpropylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-ethylisopropylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-ethylmethylcyclopropylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-(1-pyrrolidinyl)-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-trifluoromethoxy-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethylthio-de(4ζ-dimethylamino)-

pristinamycin I_A,

4ζ-methylthiomethyl-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)-pristinamycin I_A,

4ζ-acetyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_H,

4ε-dimethylamino-de(4ζ-dimethylamino)-pristinamycin I_A,

4ε-methylthio-de(4ζ-dimethylamino)-pristinamycin I_A,

4ε-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A.

The present invention is also directed towards a process which is particularly useful for preparing the compounds of the general formula I.

More precisely, it relates to a process for preparing streptogramins, characterized in that it employs a streptogramin-producing microorganism strain which possesses at least one genetic modification which affects the biosynthesis of a precursor of the group B streptogramins, and in that the said mutant strain is cultured in a culture medium which is appropriate and which is supplemented with at least one novel precursor which is differ nt from that whose biosynthesis is altered, and in that the said streptogramins are

recover d.

The strains which are employed within the scope of the present invention are therefore strains which produce streptogramins and which are mutated. The genetic modification(s) can be located either within one of the genes which is involved in the biosynthesis of the said precursors or outside the coding region, for example in the regions responsible for the expression and/or the transcriptional or post-transcriptional regulation of the said genes, or in a region belonging to the transcript containing the said genes.

According to one particular embodiment of the invention, the mutant strains possess one or more genetic modifications within at least one of their genes which is/are involved in the biosynthesis of the group B streptogramin precursors.

This or these genetic modification(s) alter(s) the expression of the said gene, that is render(s) this gene, and, as the case may be, another of the genes involved in the biosynthesis of the precursors, partially or totally incapable of encoding the natural enzyme which is involved in the biosynthesis of at least one precursor. The inability of the said genes to encode the natural proteins may be manifest d either by the production of a protein which is inactive due to structural or conformational modifications, or by the absence of production, or by

the production of a protein having an altered enzymic activity, or else by the production of the natural protein at an attenuated level or in accordanc with a desired mode of regulation. The totality of these possible manifestations is expressed by an alteration of, or perhaps a blockage in, the synthesis of at least one of the group B streptogramin precursors.

The genes which are capable of being mutated within the scope of the present invention are preferably the genes which are involved in the biosynthesis of the following precursors: L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid, L-phenylglycine and/or 3-hydroxypicolinic acid (3-HPA).

These genes are more preferably the papA, papM, papB (SEQ ID No. 3), papC (SEQ ID No. 2), hpaA (SEQ ID No. 8), snbF (SEQ ID No. 6) and pipA (SEQ ID No. 5) genes described below.

The papA and papM genes have already been described in Patent Application PCT/FR93/0923. They are present on the cosmid pIBV2. The papA gene appears to correspond to a gene for biosynthesizing 4-amino-L-phenylalanine from chorismate. The 4-amino-L-phenylalanine is then dimethylated by the product of the papM gene, an N-methyltransferase, in order to form 4-dimethylamino-L-phenylalanine, DMPAPA, which is then incorporated into pristinamycin I_A. These two genes are more particularly involv d, therefore, in the synthesis

of the precursor termed DMPAPA.

The other genes, papB, papC, pipA, snbF and hpaA, have been identified and characterized within the scope of the present invention. They are grouped together with the snbA, papA and papM genes on a chromosomal region of approximately 10 kb (Figure 7).

The sequence homologies demonstrated for the PapB and PapC proteins show that these proteins are also involved, jointly with the papA and papM proteins, in the biosynthesis of the DMPAPA precursor. The two corresponding novel genes, papB and papC, were isolated and identified by subcloning which was carried out using cosmid pIBV2, described in Patent Application PCT/FR93/0923, and a plasmid, pVRC900, which is derived from pIBV2 by means of a HindIII deletion and is also described in Patent Application PCT/FR93/0923.

The comparison of the protein encoded by the papC gene with the protein sequences contained in the Genpro library shows a 27% homology with the region which is involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of E. coli (Hudson and Davidson, 1984) and Erwinia herbicola (EMBL data library, 1991). This region of TyrA catalyses aromatization of the prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, which proceeds from 4-deoxy-4-aminoprephenate and leads to 4-aminophenylpyruvate is very probably involved in the synthesis of

DMPAPA. It would be catalysed by the PapC protein (SEQ ID No. 2).

PapB possesses a 24 to 30% homology with the region which is involved in the chorismate mutase activity of the TyrA and PheA bifunctional proteins of *E. coli* (Hudson and Davidson, 1984) and of the TyrA protein of *Erwinia herbicola*. This region catalyses isomerization of the chorismate to form prephenate in the biosynthesis of tyrosine and of phenylalanine. The PapB protein (SEQ ID No. 3) is probably involved in a similar isomerization which proceeds from 4-deoxy-4-aminochorismate and leads to 4-deoxy-4-aminoprephenate in the synthesis of DMPAPA.

The *pipA*, *snbF* and *hpaA* genes have been located in the regions which are contained between the *snbA* gene, which encodes 3-hydroxypicolinic acid AMP ligase and is described in Patent Application PCT/FR93/0923, and the *papA* or *snbR* genes. They were located accurately by means of subcloning, which was carried out using the plasmid pVRC900 and the cosmid pIBV2, which are described in Patent Application PCT/FR93/0923.

On comparing the protein encoded by the *hpaA* gene and the protein sequences contained in the Genpro library, a homology of from 30 to 40% was detected with a group of proteins which are probably involved (Thorson *et al*., 1993) in th transamination of intermediates in the biosynthesis of various

antibiotics (DnrJ, EryC1, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by another route than that of cyclodeamination (see examples 1-2 and 2-1), probably requires a transamination step which can be catalysed by the product of this gene termed hpaA (SEQ ID No. 8). Furthermore, the results of mutating this gene demonstrate unequivocally that it is involved in the synthesis of the 3-HPA precursor.

Comparison of the product encoded by the gene termed pipA with the protein sequences contained in the Genpro library shows a 30% homology with the ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step of the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of incorporating labelled lysine, that 4-oxopipecolic acid and 3-hydroxypicolinic acid, which are found both in PI_A and in virginiamycin S1, derived from lysine (Molinero et al., 1989, Reed et al., 1989). Cyclodeamination of lysine, in a similar manner to that described for ornithine, would lead to the formation of pipecolic acid. Taking this hypothesis into account, this product was termed PipA (SEQ ID No. 5). The results of mutating the pipA gene, presented in the examples below, demonstrate that it is involved solely in the synthesis of pip colic acid. It is noted, in

particular, that this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

Finally, on comparing the product of the gene termed snbF with the protein sequences contained in the Genpro library, a 30 to 40% homology was noted with several hydroxylases of the cytochrome P450 type, which are involved in the biosynthesis of secondary metabolites (Omer et al., 1990. Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of the precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The corresponding protein was termed SnbF (SEQ ID No. 6).

The results of mutating the pipA gene, with polar effects on the expression of the snbF gene, demonstrate the involvement of the snbF gene in the hydroxylation of the pipecolic acid residue of group B streptogramins. The expression of the snbF gene is thus altered by the expedient of effecting a genetic modification of the pipA gene.

Preferentially, the genetic modification(s) render(s) the said gene partially or totally incapable of encoding the natural protein.

Genetic modification should be understood to

mean, more particularly, any suppression, substitution, deletion, or addition of one or more bases in the gene(s) under consideration. Such modifications may be obtained <u>in vitro</u> (on the isolated DNA) or <u>in situ</u>, for example, by means of genetic engineering techniques, or else by exposing the said microorganisms to a treatment using mutagenic agents. Examples of mutagenic agents which may be cited are physical agents such as high-energy rays (X, γ, ultra violet, etc. rays), or chemical agents which are able to react with different functional groups of the DNA bases, and, for example, akylating agents [ethyl methanesulphonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine, and N-nitroquinoline-1-oxide (NQO)], bialkylating agents, intercalating agents, etc. Deletion is understood to mean any suppression of a part for all of the gene under consideration. This deletion can, in particular, be of a part of the region encoding the said proteins, and/or of all or part of the promoter region for transcription or translation, or else of the transcript.

The genetic modifications may also be obtained by means of gene disruption, for example using the protocol initially described by Rothstein [Meth. Enzymol. <u>101</u> (1983) 202] or, advantageously, by means of double homologous recombination. In this case, th integrity of the coding sequence will preferentially be disrupted in order to permit, if need be, replacement,

by means of homologous recombination, of the wild-type genomic sequence with a non-functional or mutant sequence.

According to another option of the invention, the genetic modifications can consist of placing the gene(s) encoding the said proteins under the control of a regulated promoter.

The mutant microorganism strains according to the present invention may be obtained from any microorganism which produces streptogramins (cf. Table V). According to one particular embodiment of the invention, the mutant strain is a strain which is derived from S. pristinaespiralis and, more particularly, from S. pristinaespiralis SP92.

Mutant strains which are preferred within the scope of the present invention and which may more particularly be mentioned are the strain SP92::pVRC508, which is mutated in the biosynthesis of the DMPAPA precursor by disrupting the papA gene by means of simple crossing over, or else, more preferably, the strain SP212, which is mutated in the biosynthesis of the DMPAPA cursor by disrupting the papA gene by means of double homologous recombination. These strains no longer produce PI unless they are supplemented with the DMPAPA precursor. Unexpectedly, when a novel precursor, which is different from DMPAPA and which is capable, after, in this cas , metabolization, of being incorporated by PI synthetase III (SnbD protein which

is responsible for incorporating L-proline and DMPAPA residues) is added to the production medium, these two strains then become able to produce novel I pristinamycins or virginiamycins, or else mainly to produce a component which is normally a minor component of PI, in particular PI_B (Figure 2).

Two other mutant strains have been prepared within the scope of the present invention. These are, respectively, the strain SP92pipA::Ωam^R, in which the pipA gene is disrupted by homologous recombination, and the strain SP92hpaA::Ωam^R, in which the hpaA gene is disrupted. While strain SP92pipA::Ωam^R no longer produces PI under standard fermentation conditions, it strongly produces, in the presence of L-pipecolic acid, a component, which was initially a minor component among the B streptogramin components, in which 4-oxopipecolic acid is replaced by L-pipecolic acid. While strain S. pristinaespiralis SP92hpaA::Ωam^R no longer produces PI under standard fermentation conditions, it is able to produce novel group B streptogramins in the presence of novel precursors.

By supplementing the medium for culturing mutant strains according to the invention with at least one novel precursor, it turns out that it is possible to orient biosynthesis either towards novel streptogramins, or towards a minor form of the streptogramins, or els to favour formation of one of the streptogramins.

The precursors which are employed within the scope of the present invention can b derivatives or analogues of amino acids and, more particularly of phenylalanine, as well as organic acids and, in particular, alpha-cetocarboxylic acids and, more particularly, derivatives of phenylpyruvic acid.

Naturally, the novel precursor is such that it caters for the alteration or blockage, which is induced in accordance with the invention, within the biosynthesis of one of the natural precursors of the group B streptogramins and leads to the synthesis of streptogramins. According to one particular embodiment of the invention, this novel precursor is selected such that it is related to the precursor whose biosynthesis is altered. Thus, in the specific case of the mutant which is blocked in the biosynthesis of DMPAPA, the novel precursor is preferably a derivative of phenylalanine.

The following may, in particular, be cited as precursors which are suitable for the invention:

Phenylalanine, 4-dimethylaminophenylalanine, 4-methylaminophenylalanine, 4-aminophenylalanine, 4-diethylaminophenylalanine, 4-ethylaminophenylalanine, 4-methylthiophenylalanine, 4-methylphenylalanine, 4-methoxyphenylalanine, 4-trifluoromethoxyphenylalanine, 4-methoxycarbonylphenylalanine, 4-chlorophenylalanine, 4-bromophenylalanine, 4-iodophenylalanine,

4-trifluoromethylphenylalanine, 4-tert-butylphenylalanine, 4-isopropylphenylalanine, 3-methylaminophenylalanine, 3-methoxyphenylalanine, 3-methylthiophenylalanine, 3-fluoro-4-methylphenylalanine, L-pipecolic acid, 4-tert-butylphenylpyruvic acid, 4-methylaminophenylpyruvic acid, 2-naphthylphenylalanine, 4-fluorophenylalanine, 3-trifluorophenylalanine, 3-ethoxyphenylalanine, 2,4-dimethylphenylalanine, 3,4-dimethylphenylalanine, 3-methylphenylalanine, 4-phenylphenylalanine, 4-butylphenylalanine, 2-thienyl-3-alanine, 3-trifluoromethylphenylalanine, 3-hydroxyphenylalanine, 3-ethylaminophenylalanine, 4-allylaminophenylalanine, 4-diallylaminophenylalanine, 4-allylethylaminophenylalanine, 4-ethylpropylaminophenylalanine, 4-ethylisopropylaminophenylalanine, 4-ethylmethylcyclopropylaminophenylalanine, 4-(1-pyrrolidinyl)phenylalanine, 4-O-allyltyrosine, 4-O-ethyltyrosine, 4-ethylthiophenylalanine, 4-ethylthiomethylphenylalanine, 4-O-(2-chloroethyl)tyrosine, 4-acetylphenylalanine, 4-ethylphenylalanine, 3-dimethylaminophenylalanine, 3-ethoxyphenylalanine, 3-fluoro-4-methylphenylalanine and 4-aminomethylphenylalanine.

Among these precursors, 4-trifluoromethoxyphenylalanin , 3-methylaminophenylalanine, 3-methylthiophenylalanine,

3-fluoro-4-methylphenylalanine, 4-methylaminophenylpyruvic acid, 3-ethoxyphenylalanine, 4-allylaminophenylalanine, 4-diallylaminophenylalanine, 4-allylethylaminophenylalanine, 4-ethylpropylaminophenylalanine, 4-ethylisopropylaminophenylalanine, 4-ethylmethylcyclopropylaminophenylalanine, 4-(1-pyrrolidinyl)phenylalanine, 4-ethylthiomethylphenylalanine, 4-O-(2-chloroethyl)tyrosine, 3-dimethylaminophenylalanine and 3-ethylaminophenylalanine are novel and were prepared and characterized within the scope of the present invention. They are found to be particularly useful for preparing streptogramins according to the invention.

The claimed process turns out to be particularly advantageous for preparing novel group B streptogramins or else for favouring formation of particular streptogramins. As such, it is particularly useful for preparing PIB.

The present invention also relates to a nucleotide sequence which is selected from among:

(a) all or part of the genes papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No. 8),

(b) sequences which hybridiz with all or part of the (a) genes, and

(c) sequences which are derived from (a) and

(b) sequences on account of the degeneracy of the genetic code.

In th particular case of the hybrid sequences according to (b), these sequences preferably encode a polypeptide which is involved in the biosynthesis of the streptogramins.

Still more preferably, the invention relates to the nucleotide sequences which are represented by the genes papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6), and hpaA (SEQ ID No. 8).

The invention furthermore relates to any recombinant DNA which encompasses a papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) or hpaA (SEQ ID No. 8) gene.

Naturally, the nucleotide sequences defined above can be part of a vector of the expression vector type, which can be an autonomously replicating vector, an integrated vector or a suicide vector. The present invention is also directed to these vectors as well as to any use of a sequence according to the invention or of a corresponding vector for, in particular, preparing metabolites of interest. It furthermore relates to any polypeptide which results from the expression of a claimed sequence.

The present inv ntion also relates to any mutated *S. pristinaespiralis* strain which possesses at least one gen tic modification within one of the papC

(SEQ ID No. 2), <u>papB</u> (SEQ ID No. 3), <u>pipA</u> (SEQ ID No. 5), <u>snbF</u> (SEQ ID No. 6) and <u>hpaA</u> (SEQ ID No. 8) genes, and, more preferably, to strains SP92<u>pipA</u>::Ω<u>am</u>^{R} and SP92<u>hpaA</u>::Ω<u>am</u>^{R}, as well as any S. <u>pristinaespiralis</u> strain, such as SP212, which possesses a genetic modification which consists of a disruption of the <u>papA</u> gene by means of double homologous recombination.

Combinations of a component of the group A streptogramins and of a compound of the general formula I, according to the invention, constitute compositions which are particularly advantageous in the therapeutic sphere. They are employed, in particular, for treating ailments which are due to Gram-positive bacteria (of the genre Staphylococci, Streptococci, Pneumococci and Enterococci) and Gram-negative bacteria (of the genre Haemophilus, Gonococci, Meningococci). Thus, the compounds according to the invention have a synergistic effect on the antibacterial action of pristinamycin IIB on Staphylococcus aureus IP8203 in mice in vivo, at oral doses which are principally between 30 mg/kg and 100 mg/kg, when they are combined in PI/PII proportions of the order of 30/70.

The present invention extends to any pharmaceutical composition which contains at least one compound of the general formula I which is or is not combined with a group A streptogramin.

The examples app aring below are present d by way of illustrating the present invention and do not

limit it.

LIST OF FIGURES.

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EXAMPLE 1: Sequencing and identification of genes involved in the biosynthesis of pristinamycin I and its precursors.

Identification, by means of sequencing, of the genes situated downstream and upstream of the gene which encodes the enzyme PapA and which is described in Patent PCT/FR93/0923, as well as of a gene which is

situated downstream of the gene which encodes the enzyme SnbA and which is also described in Patent PCT/FR93/0923.

This example describes how, using cosmid pIBV2, which is described in Patent PCT/FR93/0923 and which contains the structural genes for the enzymes PapA and PapM, which are involved in the synthesis of the 4-dimethylamino-L-phenylalanine (DMPAPA) precursor of pristinamycin I, and the structural gene for the enzyme SnbA, which is responsible for activating the aromatic precursor, 3-hydroxypicolinic acid (3-HPA), of pristinamycin I, it proved possible to identify, by sequencing around these genes and studying the corresponding mutants, other genes which are involved in the biosynthesis of the DMPAPA precursor or in the biosynthesis of other precursors of pristinamycin I.

With this aim in mind, subclonings were carried out using cosmid pIBV2 and plasmid pVRC900, which is derived from pIBV2 by means of a HindIII deletion and which is also described in Patent PCT/FR93/0923.

This example illustrates how the nucleotide sequences of fragments situated downstream and upstream of the papA and snbA genes of S. pristinaespiralis can be obtained.

The techniques for cloning DNA fragments of interest in the M13mp18 and 19 vectors (Messing et al. 1981) are standard techniques for cloning in

Escherichia coli and are described in Maniatis *et al.* (1989).

1-1 Sequencing and analysis of the region downstream of the papA gene

In order to sequence this region, which is contained between the *papA* and *papM* genes, the *Pst*I-*Pst*I fragment of 1.5 kb, the *Pst*I-*Xho*I fragment of 0.7 kb, and the *Xho*I-*Xho*I fragment of 0.7 kb were subcloned into the M13mp18 and M13mp19 vectors proceeding from plasmid pVRC900. The cloning sites were sequenced through by sequencing on double-stranded DNA using plasmids pVRC900 and pVRC409, which are described in Patent PCT/FR93/0923.

The clonings were carried out as follows. Approximately 2 µg of plasmid pVRC900 were cut with restriction enzymes *Pst*I and/or *Xho*I (New England Biolands) under the conditions recommended by the supplier. The restriction fragments thus obtained were separated on a 0.8% agarose gel, and the 1.5 kb *Pst*I-*Pst*I, 0.7 kb *Pst*I-*Xho*I and 0.7 kb *Xho*I-*Xho*I fragments of interest were isolated and purified using Geneclean (Bio101, La Jolla, California). For each cloning, approximately 10 ng of M13mp19 and/or M13mp18, cut with *Pst*I and/or *Xho*I, were ligated to 100 ng of the fragment to be cloned under the conditions described by Maniatis *et al.* 1989. After transforming the strain TG1 (K12, Δ(*lac-pro*) *supE thi hsd* ΔS F' traD36 *proA*$^{+}$*B*$^{+}$ *lacI*^{q} *lacZ* Δ M15; Gibson, 1984) and

selecting lysis plaques on an LB + X-gal + IPTG medium in accordance with the technique described by Maniatis et al. (1989), the phage carrying the desired fragments were isolated. The different inserts were sequenced by the chain termination reaction using, as the synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. The reactions were carried out using fluorescent dideoxynucleotides (PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit-Applied Biosystem) and analysed on a model 373 A Applied Biosystems DNA sequencer. The overlap between these different inserts was such that it was possible to establish the entire nucleotide sequence between the papA and papM genes (SEQ ID No. 1).

With the aid of this nucleotide sequence, it is possible to determine the open reading frames and thereby identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of PI or its precursors, as well as the polypeptides encoded by these genes.

We looked for the presence of open reading frames within the 2.9 kb PstI-XhoI fragment, which contains the nucleotide sequence between the papA and papM genes, making use of the fact that Streptomyces DNA displays a high percentage of G and C bases as well as a strong bias in the use of codons which make up the

coding fram s (Bibb et al. 1984). The method of Staden and McLachlan (1982) makes it possible to calculate the probability of coding frames in terms of th codon usage of Streptomyces genes which have already been sequenced and which are assembled in a data file which contains 19673 codons and which was obtained using the BISANCE (Dessen et al. 1990) computer server.

Using this method, it was possible to characterize four highly probable open reading frames within the 2.9 kb PstI-XhoI fragment, which reading frames are depicted in the table below (TABLE I). They are designated frames 1 to 4 according to their position starting from the PstI site. The length of each reading frame in bases, has been indicated, as has its position within the fragment (the PstI site being situated at position 1); the number of amino acids in the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 1, 3 and 4 are encoded by the same strand, while frame 2 is encoded by the complementary strand (Figure 4). Frames 1 and 4 correspond, respectively, to the C-terminal region of the PapA protein and to the N-terminal region of the PapM protein, which proteins were previously identified and described in Patent PCT/FR93/00923.

Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
1 (PapA)	1-684	684	-
2 (PapC)(inv)	949-1836	888	296
3 (PapB)	1873-2259	387	129
4 (PapM)	2259-2887	629	-

TABLE I

Comparison of the product of frame 2 (TABLE I) with the protein sequences contained in the Genpro library shows a 27% homology with the region involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of *E. coli* (Hudson and Davidson, 1984) and of *Erwinia herbicola* (EMBL data library, 1991). This region of TyrA catalyses aromatization of prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, proceeding from 4-deoxy-4-aminoprephenate and leading to 4-aminophenylpyruvate is very probably involved in the synthesis of DMPAPA. This reaction will be catalysed by the product of frame 2, t rmed PapC (SEQ ID N . 2).

Comparison of the product of frame 3 (TABLE I) with the protein sequences contained in the

Genpro library shows a 24 to 30% homology with the region involved in the chorismate mutase activity of the bifunctional TyrA and PheA proteins of E. coli (Hudson and Davidson, 1984) and of the TyrA protein of Erwinia herbicola. This region catalyses isomerization of chorismate to form prephenate in the biosynthesis of tyrosine and phenylalanine. A similar isomerization, proceeding from 4-deoxy-4-amino chorismate and leading to 4-deoxy-4-aminoprephenate, is very probably involved in the synthesis of DMPAPA. This reaction would be catalysed by the product of frame 3, termed PapB (SEQ ID No. 3).

In the case of TyrA and PheA, the chorismate mutase and prephenate dehydratase, or prephenate dehydrogenase, activities are catalysed by the same protein. In S. pristinaespiralis, the chorismate mutase and prephenate dehydrogenase enzyme activities are catalysed by two separate proteins, i.e. PapB and PapC, respectively.

The sequence homologies demonstrated for the PapB and PapC proteins demonstrate that these two proteins are involved, jointly with the PapA and PapM proteins, in the biosynthesis of the aromatic derivative DMPAPA. In the same way as for papA, disruption of the papB and papC genes should lead to the construction of S. pristinaespiralis strains which are incapable of producing PI but which are able, in the presence of novel precursors, to produce new PIs

which are modified at the level of the DMPAPA residue.

1-2. Sequencing and analysis of the region upstream of the papA gene

This region is contained between the snbA gene, which encodes 3-hydroxypicolinic acid AMP ligase and which is described in Patent PCT/FR93/00923, and the papA gene.

The clonings were carried out as described in Example 1-1, proceeding from plasmid pVRC900 and cosmid pIBV2, which are described in Patent PCT/FR93/00923. The 1.3 kb XhoI-XhoI, 0.2 kb XhoI-XhoI, 3.3 kb XhoI-XhoI, 1.1 kb HindIII-PstI and 2.2 kb PstI-PstI fragments were subcloned into the M13mp18 and M13mp19 vectors. These different clonings made it possible to pass through all the cloning sites. The different inserts were sequenced as described in 1-1 using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence in the insert to be sequenced.

The overlap between these different inserts enabled the entire nucleotide sequence which is present between the snbA and papA genes (SEQ ID No. 4) to be established.

On the basis of this nucleotide sequence, it is possible to determine the open reading frames and to identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of precursors of PI, as well as the polypeptides encoded by these

genes.

We have looked for the presence of open reading frames within the 4.5 kb XhoI-PstI fragment, which contains the nucleotide sequence between the snbA and papA genes, as described in Example 1.1. Using this method, it was possible to characterize four highly probable open reading frames within the 4.5 kb XhoI-PstI fragment, which frames are depicted in the table below (TABLE II). They are designated frames 1 to 4 in accordance with their position starting from the XhoI site. Their length in bases, and their position within the fragment (the XhoI site being situated at position I) has been indicated for each fragment; the number of amino acids within the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 2, 3 and 4 are encoded by the same strand, and frame 1 is encoded by the complementary strand (Figure 5). Frames 1 and 4 correspond, respectively, to the N-terminal regions of the SnbA and PapA proteins, which were previously identified and described in patent PCT/FR93/00923.

Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
1 (SnbA)(inv)	1-329	329	-
2 (PipA)	607-1671	1065	355
3 (SnbF)	1800-2993	1194	398
4 (PapA)	3018-4496	1479	-

TABLE II

Comparison of the product of frame 2 (TABLE II) with the protein sequences contained in the Genpro library shows a 30% homology with ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step in the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of the incorporation of labelled lysine, that 4-oxopipecolic acid and 3-hydroxypicolinic acid, which are found both in PI_A and in virginiamycin S1, derived from lysine (Molinero et al., 1989; Reed et al., 1989). A reaction in which lysine was cyclodeaminated, similar to that described for ornithine, would lead to the formation of pipecolic acid. Taking this hypothesis into account, the product of frame 2 was termed PipA

(SEQ ID No. 5). The results of mutating the pipA gene, presented in 2-1, demonstrate that the pipA gene is involved solely in the synthesis of pipecolic acid, since this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

Comparison of the product of frame 3 (TABLE II) with the protein sequences contained in the Genpro library shows a 30 to 40% homology with several hydroxylases of the cytochrome P450 type, which hydroxylases are involved in the biosynthesis of secondary metabolites (Omer et al., 1990, Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The results of mutating the pipA gene, presented in 2-1-3, demonstrate that the product of frame 3 is involved in hydroxylation of the pipecolic acid residue of PI_B. The corresponding gene has therefore been termed snbF, and the corresponding protein SnbF (SEQ ID No. 6).

1-3. Sequencing the region downstream of the snbA gene.

This region is included between the snbA gene, which encodes 3-hydroxypicolinic acid adenylate

ligase, and the snbR gene, which encodes a membrane protein which is probably responsible for transport and for resistance to PI, with both genes having been described in Patent PCT/FR93/00923. Sequencing of this region was carried out using a fragment which was isolated from cosmid pIBV2, as described in Example 1-1.

The 1.6 kb HindIII-BglII fragment was subcloned into the M13mp18 and M13mp19 vectors, proceeding from cosmid pIBV2. The insert was sequenced as described in 1-1, using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. On the basis of the nucleotide sequence thus obtained (SEQ ID No. 7), it is possible to determine the open reading frames and to identify, in *S. pristinaespiralis*, genes which are involved in the biosynthesis of the precursors of PI, as well as the polypeptides encoded by these genes. We looked for the presence of open reading frames within the 1.6 kb HindIII-BglII fragment, which corresponds to the end of the snbA gene and its downstream region, as described in Example 1-1. A complete open coding frame, encoded by the same strand as the snbA gene (Figure 6), was detected. Relative to position 1, corresponding to the HindIII site, this frame starts at nucleotide 249, i.e. 30 nucleotides after the end of the snbA gene, and terminates at nucl otide 1481. It is 1233 nucleotides

in size, corresponding to a protein of 411 amino acids.

Comparison of the product of this open frame with the protein sequences contained in the Genpro library shows a 30 to 40% homology with a group of proteins which are probably involved (Thorson et al., 1993) in the transamination of intermediates in the biosynthesis of various antibiotics (DnrJ, EryC1, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by a route other than cyclodeamination (see Examples 1-2 and 2-1), could necessitate a transamination step which can be catalysed by the product of this frame 3, termed HpaA (SEQ ID No. 8). The results of mutating this gene, presented in 2-2, demonstrate unequivocally that this gene is involved in synthesis of the 3-HPA precursor and confirm our hypothesis.

The genes papB, papC, pipA, snbF and hpaA, which are described in the present invention, are grouped together with the snbA, papA and papM genes on a chromosomal region of approximately 10 kb (Figure 7). This confirms the presence of a cluster of genes which are involved in the biosynthesis of PI and its precursors. Studying regions upstream and downstream of this cluster should enable the other genes involved in the biosynthesis of PI precursors, in particular L-phenylglycine and L-2-aminobutyric acid, to be identified.

EXAMPLE 2: Construction of recombinant strains by means of disrupting identified genes.

This example illustrates how it is possible to demonstrate involvement of the genes described in Example 1 in the biosynthesis of pristinamycin precursors, and also to construct S. pristinaespiralis strains which are able to produce novel pristinamycins. These strains are obtained by disrupting the genes which are involved in the biosynthesis of the residue which it is desired to replace, and the novel pristinamycins are produced by supplementing these mutants with novel precursors.

Strain SP92::pVRCC508, which is employed in the present invention to produce novel derivatives of PI by replacing the precursor DMPAPA with other molecules, is described in Patent PCT/FR93/0923. It is obtained by disrupting, by means of simple crossing over, the papA gene, which is involved in the biosynthesis of the precursor of DMPAPA and is thought to participate in an early step relating to the transamination of chorismate. This disruption has a polar character since, in this mutant, expression of the papM gene (PCT/FR93/0923), which is situated 1.5 kb downstream of the papA gene and is involved in the double methylation of 4-amino-L-phenylalanine to form DMPAPA, is very reduced. Thus, assaying the activity of the SAM-dependant methylation enzyme for converting 4-amino-L-phenylalanine (PAPA) into DMPAPA indicates that

mutant SP92::pVRC508 has an activity which is less than 5% of the activity of the wild-type strain.

In the present invention, this strain, SP92::pVRC508, can be used, under appropriate fermentation conditions and supplementation conditions, to produce novel pristinamycins which are modified at the level of the DMPAPA residue, as will be explained in Example 3. Mutants having the same phenotype can be obtained by disrupting the papB or papC genes described in the present invention.

Another type of S. pristinaespiralis strain, whose papA gene is disrupted and which possesses the same phenotype as strain SP92::pVRC508, was obtained in a similar manner by disrupting the papA gene by means of double crossing over. This construction was carried out starting with a 4.6 kb SphI-HindIII fragment, which fragment was isolated from cosmid pIBV2 and contains the 3' region of the pipA gene, the entire snbF and papA genes and the 3' part of the papC gene. This fragment was cloned into the suicide vector pDH5, which vector is only able to replicate in E. coli but carries a resistance marker which is expressed in Streptomyces (the gene for resistance to thiostrepton or to nohiheptide, tsr). This vector, pDH5, was developed by Wohlebben et al (1991 Nucleic Acid Res. 19, 727-731). A BclI-BclI deletion of 1.1 kb was then made in the papA gene, and a 2.2 kb HindIII-HindIII fragment, carrying the amR gene (resistance to geneticin and to

apramycin), was introduced after the cohesive ends had been filled in. The recombinant vector was termed pVRC414 and is depicted in Figure 12. After transforming the pristinamycin-producing strain with plasmid pVRC414, transformants which were resistant to geneticin and sensitive to thiostrepton were isolated and analysed. These clones are the result of a double homologous recombination between the S. pristinaespiralis DNA regions of plasmid pVRC414 and the corresponding chromosomal region of S. pristinaespiralis, as described in Figure 13. One of these clones was termed SP212. Its phenotype is identical to that of strain SP92::pVRC508 as regards the absence of any production of PI and the ability of the strain to produce new antibiotics in the presence of novel precursors. Advantageously, this type of strain, which is obtained by double crossing over, is more stable than the strains which are obtained by simple crossing over.

2-1. Construction of a mutant of S. pristinaespiralis SP92 whose pipA gene is disrupted.

This example illustrates how it is possible, by means of disrupting the pipA gene, to construct a strain of S. pristinaespiralis SP92 which no longer produces PI under standard fermentation conditions and which is able to produc new pristinamycins, which are modified at the level of the 4-oxopipecolic acid residue of PIA, when novel precursors are added to the

fermentation.

It was constructed using a suicide vector, the vector pUC1318, which only replicates in *E. coli.* This vector does not carry any resistance marker which is expressed in Streptomyces. Its presence in the genome of Streptomyces can only be detected by colony hybridization.

2-1-1. Construction of plasmid pVRC420:

This example illustrates how it is possible to construct a plasmid which does not replicate in *S. pristinaespiralis* SP92 and which can be employed to disrupt the *pipA* gene by means of double homologous recombination.

Plasmid pVRC420 was constructed in order to produce the chromosomal mutant of SP92 in which the *pipA* gene is disrupted, proceeding from cosmid pIBV2, which is described in Patent PCT/FR93/0923. Cosmid pIBV2 was cut with the restriction enzyme *Pst*I and, after the fragments, thus generated, had been separated by electrophoresis on a 0.8% agarose gel, a 2.8 kb *Pst*I-*Pst*I fragment, containing the start of the *snbA* and *snbF* genes and the whole of the *pipA* gene, was isolated and purified using Geneclean (Bio101, La Jolla, California). 50 ng of vector pUC1318, which had been linearized by digesting with *Pst*I, were ligated to 200 ng of the 2.8 kb fragment, as described in Example 1. A clone carrying the desired fragment was isolated following transformation of the strain TG1 and

selection on LB + 150 µg/ml ampicillin + X-gal + IPTG medium. The recombinant plasmid was termed pVRC415 (Figure 8). A cassette containing the am^R gene, encoding resistance to apramycin or to geneticin (Kuhstoss et al., 1991), was then introduced into the unique HindIII site of plasmid pVRC415, this site being situated 530 bp downstream of the start of the pipA gene. This construction was effected as follows. A 2.5 kb DNA fragment, containing the am^R gene, the PermE promoter (Bibb et al., 1985) and the first 158 amino acids of the gene for resistance to erythromycin , ermE, was isolated by means of a SalI-BglII double digestion of a plasmid which was derived from plasmids pIJ4026 (plasmid carrying the ermE gene under the control of the PermE promoter) and pHP45Ωam^R. After filling in the SalI and BglII protruding 5′ cohesive ends using Klenow enzyme in accordance with the protocol described by Maniatis et al., 1989, the fragment containing the am^R gene was cloned into the HindIII site of plasmid pVRC415, whose protruding 5′ cohesive ends had also been filled in with Klenow enzyme as previously described. The recombinant plasmid thus obtained was designated pVRC420. Its restriction map is depicted in Figure 9.

2-1-2. Isolation of mutant SP92pipA::Ωam^R, whose pipA gene is disrupted by homologous recombination.

This example illustrates how the mutant of

S. pristinaespiralis SP92 whose pipA gene is disrupted was constructed.

This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC420.

The preparation of protoplasts, their transformation and extraction of the total DNA from the recombinant strains were all effected as described by Hopwood et al. (1985).

The strain SP92 was cultured, at 30°C for 40 hours, in YEME medium (Hopwood et al., 1985), 34% sucrose, 5 mM $MgCl_2$ and 0.25% glycine. The mycelium was protoplasted in the presence of lysozyme, and 5 × 1 µg of pVRC420 were used to transform (by the method employing PEG) the protoplasts. After one night in which the protoplasts were regenerated on R2YE medium (D. Hopwood et al. 1985), the recombinants were selected by spreading on 3 ml of SNA medium (D. Hopwood et al. 1985) containing 1,500 µg/ml geneticin.

100 clones which were resistant to geneticin were isolated from the 5 transformations that were carried out. These recombinants arise from integration, by means of simple or double homologous recombination between the pipA gene which is carried by the chromosome of strain SP92 and the parts of the pipA gene which are contained in the 5.3 kb fragment carried by the suicide plasmid pVRC420. In order to select the recombinants which were obtained by double crossing over (that is which did not contain the pUC1318 part of

plasmid pVRC420 in their genome), colony hybridizations were carried out on 90 clones using pUC19 labelled with [α-^{32}P]dCTP as the probe, as described in Maniatis et al (1989). 10 clones were selected which were resistant to geneticin but which did not hybridize the vector pUC19. The spores of the recombinants were isolated by streaking and growing on HT7 medium containing 10 μg/ml geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the position at which plasmid pVRC420 was integrated, various Southerns of the total DNA from several recombinant clones, purified as described by Hopwood et al. 1985, were carried out, with hybridization to the 2.8 kb PstI-PstI fragment, which was used as a probe after having been labelled with [α-^{32}P]dCTP. The results confirm that these recombinants were obtained by double crossing over between vector pVRC420 and the chromosome of strain SP92, resulting in replacement of the 2.8 kb PstI-PstI fragment, containing the pipA gene, by a 5.3 kb PstI-PstI fragment containing the pipA gene which is disrupted by introduction of the am^R gene. One of these mutants was designated SP92pipA::Ωam^R.

2-1-3. Production of pristinamycins using mutant SP92pipA::Ωam^R.

This example illustrates how it is establish d that the mutant of S. pristinaespiralis SP92 whose pipA gene is disrupted by int gration of plasmid pVR420 on the one hand no longer produces PI

under standard fermentation conditions and on the other hand exhibits a high 1 vel of production of a minor form of the B components of streptogramins in which 4-oxopipecolic acid is replaced by pipecolic acid.

Mutant SP92<u>pip</u>A::Ω<u>am</u>^{R}, as well as strain SP92 in the role of a control strain, were cultured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the abovementioned strain are added, under sterile conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of 10 g/l corn steep, 15 g/l sucrose, 10 g/l $(NH_4)_2SO_4$, 1 g/l K_2HPO_4, 3 g/l NaCl, 0.2 g/l $MgSO_4$-$7H_2O$ and 1.25 g/l $CaCO_3$. The pH is adjusted to 6.9 using sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 hold, are added under sterile conditions to 30 ml of production medium in a 300 ml Erlenmeyer flask. The production medium is made up of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before introducing the calcium carbonate. The Erlenmeyer flasks are shaken for 24, 28 and 32 hours at 27°C. At each time point, 10 g of must are weighed into a smooth Erlenmeyer flask to which 20 ml of mobile phase,

consisting of 34% of acetonitrile and 66% of a solution of 0.1 M KH_2PO_4 (adjusted to pH 2.9 with concentrat d H_3PO_4) are added for extracting the pristinamycins. After shaking, the whole is centrifuged and the pristinamycins contained in the supernatant are assayed by HPLC by means of injecting 150 µl of the centrifugation supernatant onto a Nucleosil 5-C8 column of 4.6 × 150 mm, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9. The I pristinamycins are detected by means of their UV absorbance at 206 nm.

The results demonstrated that, under the fermentation conditions employed, mutant SP92<u>pip</u>A::Ω<u>am</u>^{R} did not produce PI at 24, 28 or 32 hrs of fermentation, while control strain SP92 produced a quantity of PI which was standard for the 3 times which were tested. The quantity of PII which was produced remained the same for the two strains. Mutant SP92<u>pip</u>A::Ω<u>am</u>^{R} is definitely blocked at a step in the biosynthesis of PI. Fermentation complementation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. The results of these complementations demonstrated that when 100 mg/l pipecolic acid and 100 mg/l DMPAPA are added simultaneously to the fermentation medium, the mutant produces what is normally a minor derivative of PI, i.e. PI_E (which is produced by SP92 in a quantity which is less than 5%)

at a level which is equivalent to the production of PI_A by the control strain. This production does not take place if the pip colic acid and the DMPAPA are added separately. PI_B differs from PI_A (major component of PI) in the absence of the keto function in the 4 position on the pipecolic acid. The fact that mutant SP92pipA::Ωam^R can only be complemented by adding pipecolic acid and DMPAPA simultaneously indicates that the papA, and probably the papB and papM genes were disrupted by a polar effect of the construct. Thus, all these genes are situated downstream of pipA and are probably cotranscripts together with pipA. Disruption of the latter therefore leads to disruption of the pap genes and, consequently, absence of DMPAPA synthesis. The fact that complementation of mutant SP92pipA::Ωam^R with pipecolic acid results in the production of PI_B and not PI_A leads to two conclusions: the first is that construction of the PI cycle is achieved by incorporating pipecolic acid and not 4-oxopipecolic acid and that a hydroxylation generating the keto function in the 4 position then takes place subsequently. The second is that this hydroxylation is probably carried out by the enzyme SnbF whose structural gene is situated directly downstream of the pipA gene. Thus, the obvious polarity of the disruption of the pipA gene on the pap genes probably involves a polar effect on the snbF gene, which is situated between pipA and the pap genes, which is manifested in

inhibition of the function of hydroxylation of the pipecolic acid residue of PI_B to form 4-hydroxypipecolic acid, which is found in PI_F and PI_G (Figure 2) and then oxidized to 4-oxopipecolic acid in PI_A.

Preparing a mutant of this nature made it possible to construct a strain of S. pristinaespiralis which is unable to produce PI except in the presence of the PI precursors DMPAPA and pipecolic acid, using which it is able to produce, in a quantity equivalent to that of the starting strain, what is normally a minor derivative of PI within the pristinamycin mixture. Similarly, in the presence of novel precursors, or of a mixture of novel precursors and of precursors which are normally present in PI, this strain will be able to produce new pristinamycins which are modified in either DMPAPA or 4-oxopipecolic acid or in both these residues.

2-2. Construction of a mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted.

This example illustrates how it is possible, by means of disrupting the hpaA gene, to construct a strain of S. pristinaespiralis SP92 which no longer produces PI under standard fermentation conditions and which is able to produce new pristinamycins, which are modified at the level of the 3-HPA precursor, when novel precursors are added to the fermentation.

This mutant was constructed using a plasmid which does not replicate in S. pristinaespiralis SP92

and which can be used for disrupting the hpaA gene by means of double homologous recombination.

2-2-1. Construction of the suicide plasmid pVRC421

Plasmid pVRC421 was constructed using a suicide vector which, while only being able to replicate in E. coli, carries a resistance marker which is expressed in Streptomyces, i.e. the gene for resistance to thiostrepton or to nosiheptide, tsr. This vector, pDH5, was developed by Hillemann et al. (1991).

Plasmid pVRC421 was constructed in order to produce the chromosomal mutant of SP92 whose hpaA gene is disrupted, making use of cosmid pIBV2, which is described in Patent PCT/FR93/0923. pIBV2 was digested with the restriction enzyme SphI and, after having separated the fragments, thus generated, by means of electrophoresis on a 0.6% agarose gel, a 4.8 kb SphI-SphI fragment, containing the whole of the hpaA gene and virtually the whole of the snbA gene, was isolated and purified using Geneclean as described above. 50 ng of the vector pDH5, linearized by digesting with SphI, were ligated to 200 ng of the 4.8 kb fragment, as subsequently described. A clone harbouring the desired fragment was isolated after transforming the strain TG1 and selecting on LB + 150 μg/ml ampicillin + IPTG + X-gal medium. The recombinant plasmid was designated pVRC411 (Figure 10). A cassette containing the gene am^R, encoding r sistance

to apramycin or to geneticin, was then introduc d into the unique PflmI site of plasmid pVRC411, this site being situated 610 bp downstream of the start of the hpaA gene. This construct was produced as follows. A 2.2 kb DNA fragment, containing the am^R gene, was isolated following digestion of the plasmid pHP45Ωam^R, containing the am^R gene, with HindIII. After filling in the HindIII protruding 5′ cohesive ends using Klenow enzyme according to the protocol described by Maniatis et al. 1989, the fragment containing the am^R gene was cloned into the PflmI site of plasmid pVRC411, whose protruding 3′ cohesive ends had been rendered blunt using the enzyme T4 polymerase as described in Maniatis et al. 1989. The recombinant plasmid thus obtained was termed pVRC421. Its restriction map is depicted in Figure 11.

2-2-2. Isolation of mutant SP92hpaA::Ωam^R, whose hpaA gene is disrupted by means of homologous recombination.

This example illustrates how the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted was constructed.

This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC421.

The protoplasts were prepared and transformed as described pr viously.

Strain SP92 was cultured, at 30°C for 40 hours, in YEME medium, 34% sucros , 5 mM $MgCl_2$, 0.25%

glycine. Th mycelium was protoplasted in the presence of lysozyme, and 5 × 1 μg of pVRC421 were employed for transforming (by the method using PEG) the protoplasts. After one night for regenerating the protoplasts on R2YE medium, the recombinants were selected by spreading on 3 ml of SNA medium containing 1,500 μg/ml geneticin.

600 clones which were resistant to geneticin were isolated from the 5 transformations which were carried out. These recombinants result from integration by means of simple or double homologous recombination between the hpaA gene carried by the chromosome of strain SP92 and the 6 kb fragment of the suicide plasmid pVRC421. In order to select the recombinants obtained by double crossing over (that is, the clones which no longer contain, in their genome, the pDH5 moiety of plasmid pVRC421), the clones were subcultured on HT7 medium containing 400 μg/ml thiostrepton. 6 clones which were resistant to geneticin but sensitive to thiostrepton were selected. The spores of the recombinants were selected by streaking and growth on HT7 medium containing 10 μg/ml geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the position of integration of plasmid pVRC421, various Southerns of the total DNA from the 6 recombinant clones, purified as described by Hopwood et al. 1985, were carried out with hybridization to the 4.8 kb SphI-SphI fragment,

which was used as the probe after having been labelled with [α-^{32}P]dCTP. Th results confirm that these recombinants were obtained by double crossing over between the vector pVRC421 and the chromosome of the SP92 strain, resulting in replacement of the 4.8 kb SphI-SphI fragment, containing the hpaA gene, by a 6 kb SphI-SphI fragment which contains the hpaA gene disrupted by the am^R gene. One of these mutants was designated SP92hpaA::Ωam^R.

2-2-3. Production of pristinamycins by mutant SP92hpaA::Ωam^R.

This example illustrates how it is established that the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted by integration of plasmid pVR421 no longer produces PI under the standard fermentation conditions.

Mutant SP92hpaA::Ωam^R, and also strain SP92 in the role of control strain, were cultured in liquid production medium. The fermentation was carried out as described in Example 2-1-3, and the pristinamycins were then extracted and assayed as previously described. The results demonstrated that, under the fermentation conditions employed, mutant SP92hpaA::Ωam^R did not produce PI, either at 24, 28 or 32 hrs of fermentation, whereas the control strain produced a quantity of PI which was standard for the 3 time points tested. The quantity of PII produced remained the same for the two strains. Mutant SP92hpaA::Ωam^R is definitely blocked at

a step in the biosynthesis of PI. Complem ntary fermentation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. When 100 mg/l 3-hydroxypicolinic acid are added to the fermentation medium, the mutant then produces PI_A at a level which is equivalent to the production of PI by the control strain. The fact that mutant SP92hpaA::Ωam^R can only be complemented by adding 3-hydroxypicolinic acid demonstrates that the hpaA gene is involved in the synthesis of this precursor.

Construction of this mutant made it possible to produce a strain of S. pristinaespiralis which is mutated as regards its production of PI but which, in the presence of the precursor 3-HPA, is capable of producing PI in a quantity equivalent to that produced by the starting strain. In the same way as in the preceding examples, it can be envisaged that it should be possible, using a mutant of this nature in the presence of novel precursors, to produce new pristinamycins which are modified at the level of the 3-hydroxypicolinic acid residue.

EXAMPLE 3: Production of compounds of the general formula I by the mutant SP92::pVRC508.

This example illustrates how the mutant of S. pristinaespiralis SP92 whose papA gene is disrupted by int gration of plasmid pVRC508 is able to synth size new streptogramins in the presence of precursors which

ar added to the production medium. These precursors can be derivatives of amino acids and, more particularly, of phenylalanine, but also of α-ketocarboxylic acids and, more particularly, of phenylpyruvic acid.

The mutant SP92::pVRC508 was cultured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the previously mentioned strain is added, under sterile conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of 10 g/l corn steep, 15 g/l sucrose, 10 g/l $(NH_4)_2SO_4$, 1 g/l K_2HPO_4, 3 g/l NaCl, 0.2 g/l $MgSO_4$-$7H_2O$ and 1.25 g/l $CaCO_3$. The pH is adjusted to 6.9 with sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 h old, are added, under sterile conditions, to 30 ml of production medium in a 300 ml Erlenmeyer flask. The production medium consists of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C on a rotating shaker at a speed of 325 rpm. After 16 h, 1 ml of a solution of one of the precursors listed in Table 3 (gen rally 5 or

10 g/l) is added to the culture. The latter is terminated 8 or 24 h later. The volume of the must is m asured immediately, and 2 volumes of mobil phase, consisting of 34% acetonitrile and 66% of a solution of 0.1 M KH_2PO_4 (adjusted to pH 2.9 with concentrated H_3PO_4) are added to it for extracting the pristinamycins. After shaking, the whole is centrifuged and the pristinamycins contained in the supernatant are extracted and purified as described in Example 4. They are also assayed by HPLC by means of injecting 150 µl of the centrifugation supernatant onto a Nucleosil 5-C8 4.6 × 150 mm column, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9. The new I pristinamycins are detected by means of their UV absorbance at 206 nm and, where appropriate, by means of their fluorescence emission (370 nm filter, excitation at 306 nm).

PRECURSOR	ORIGIN
ph nylalanine	Janssen
4-dimethylaminophenylalanine	Example 33
4-methylaminophenylalanine	Example 34-1
4-aminophenylalanine	Janssen 22.794.96
4-diethylaminophenylalanine	Example 33
4-ethylaminophenylalanine	Example 33
4-methylthiophenylalanine	Example 33
4-methylphenylalanine	J.P.S101-312-4/ Example 33
4-methoxyphenylalanine	Janssen 16.975.97
4-trifluoromethoxyphenylalanine	Example 34-8
4-methoxycarbonylphenylalanine	Example 33
4-chlorophenylalanine	Janssen 15.728.14
4-bromophenylalanine	Janssen 22.779.81
4-iodophenylalanine	Bachem F 1675
4-trifluoromethylphenylalanine	P.C.R. Inc. 12 445-3
4-tert-butylphenylalanine	Example 35-1

4-isopropylphenylalanine	Example 36-1
3-methylaminophenylalanine	Example 35-3
3-methoxyphenylalanine	J.P.S. 101-313-2
3-methylthiophenylalanine	Example 34-11
3-fluoro-4-methylphenylalanine	Example 34-5
4-tert-butylphenylpyruvic acid	Example 33
4-methylaminophenylpyruvic acid	Example 34-4
2-napthylphenylalanine	Bachem F 1865
4-fluorophenylalanine	Bachem F 1535
PRECURSOR	**ORIGIN**
3-fluorophenylalanine	Bachem F 2135
3-ethoxyphenylalanine	Example 37-1
2,4-dimethylphenylalanine	Example 33
3,4-dimethylphenylalanine	Example 33
3-methylphenylalanine	Example 33
4-phenylphenylalanine	Example 33
4-butylphenylalanine	Example 36-3
2-thienyl-3-alanine	Aldrich 28.728.8
3-trifluoromethylphenylalanine	Example 33
3-hydroxyphenylalanine	Aldrich T 9.039.5
3-ethylaminophenylalanine	Example 35-6

4-aminomethylphenylalanine	Example 33
4-allylaminophenylalanin	Example 38-2
4-diallylaminophenylalanine	Example 38-1
4-allylethylaminophenylalanine	Example 39-4
4-ethylpropylaminophenylalanine	Example 39-6
4-ethylisopropylaminophenylalanine	Example 39-1
4-ethylmethylcyclopropylamino-phenylalanine	Example 39-8
4-(1-pyrrolidinyl)phenylalanine	Example 40-1
4-O-allyltyrosine	Example 33
4-O-ethyltyrosine	Example 33
4-ethylthiophenylalanine	Example 33
4-ethylthiomethylphenylalanine	Example 41-1
4-O-(2-chloroethyl)tyrosine	Example 42-1
4-acetylphenylalanine	Example 33
4-ethylphenylalanine	Example 33
3-dimethylaminophenylalanine	Example 35-10

TABLE III

The following table (TABLE IV) indicates th relative retention times of the new PI which are produc d, taking PI_A as th reference. The absolute

retention times were determined at 25°C in the HPLC system described above; th y vary slightly from one injection to another and also in accordanc with temperature.

Precursor	t_R (relative retention time) of the new PI (Neo PI)		
	Neo PI_A	Neo PI_B	Other neo PI
4-methylaminophenylalanine	0.85		
4-aminophenylalanine	0.64		
4-methylthiophenylalanine	1.93	2.73	1.63
4-methylphenylalanine	1.77	2.65	
4-methoxyphenylalanine	1.46		
4-methoxycarbonylphenyl-alanine	1.49		
4-chlorophenylalanine	2.04		
4-bromophenylalanine	2.16		
4-iodophenylalanine	2.42		
4-trifluoromethylphenyl-alanine	2.56	3.74	
4-tert-butylphenylalanine	3.34		

4-isopropylphenylalanin	2.80		4.35
3-methylaminophenylalanine	1.15		
3-methoxyphenylalanine	1.49	2.04	
3-fluoro-4-methylphenylalanine	2.93		
4-tert-butylphenylpyruvic acid	3.34		
4-methylaminophenylpyruvic acid	0.85		
4-ethylaminophenylalanine	0.94		
4-diethylaminophenylalanine	0.61		
4-allylaminophenylalanine	1.83		
4-diallylaminophenylalanine	2.64		
4-allylethylaminophenyl-alanine	2.4		
4-ethylpropylaminophenyl-alanine	1.06		
4-ethylisopropylamino-phenylalanine	0.89		
4-ethylmethylcyclopropyl-aminophenylalanine	1.1		

4-(1-pyrrolidinyl)phenyl-alanine	2.0		
4-O-trifluoromethyltyrosine	2.42		
4-O-allyltyrosine	2.62		
4-O-ethyltyrosine	2.2		
4-ethylthiophenylalanine	1.96		
4-methylthiomethylphenyl-alanine	1.98		
4-O-(2-chloroethyl)tyrosine	2.45		
4-acetylphenylalanine	1.61		
4-ethylphenylalanine	1.86	2.40	
3-dimethylaminophenyl-alanine	1.49		
3-methylthiophenylalanine	1.93		
3-O-ethyltyrosine	1.78		

TABLE IV

The new PI, with a t_R of 4.35, for 4-isopropylphenylalanine corresponds to a neo PI_E which is d scribed in Example 14.

The new PI, with a t_R of 1.63, for 4-methylthiophenylalanine corresponds to a 5γ-hydroxy

neo PI_B, which is described in Example 5.

The mutant SP92::pVRC508 was otherwise ferment d in the pres nc of 4-dimethylaminophenylalanine. Under these conditions of complementation, mutant SP92::pVRC508 produces a quantity of I_A pristinamycins which is equivalent to that produced by strain SP92.

EXAMPLE 4: Preparation of pristinamycin I_B [4ζ-methylamino-de(4ζ-dimethylamino)pristinamycin I_A] and of 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A

4.1: Preparation of pristinamycin I_B [4ζ-methylamino-de)4ζ-dimethylamino)pristinamycin I_A]

The strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as described in Example 3, with 1 ml of a 10 g/l aqueous solution of (R,S)-4-methylaminophenylalanine, synthesized as in Example 34-1, being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry xtract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichlormethane and is successively eluted

with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing pristinamycin I_B ar combined and evaporated. The dry residue is taken up in 6 ml of a mixture of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture of 65% 100 mM phosphate buffer, pH 2.9, and 35% acetonitrile. The fractions containing pristinamycin I_B are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried on sodium sulphate and then evaporated. 52 mg of pristinamycin I_B are obtained.

NMR spectrum. ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.71 (dd, J=16 and 6 Hz, 1H, 5 β_2), 0.92 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.10 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.34 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.03 (mt, 1H, 3 β_1), 2.22 (mt, 1H, 5 δ_2), 2.33 (broad d, J=16 Hz, 1H: 5 δ_1), 2.40 (d, J=16 Hz, 1H: 5 β_1), 2.82 (mt, 1H: 5 ϵ_2), 2.81 (s, 3H: 4 NCH_3 in the para position of the phenyl), 2.90 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.29 (s, 3H: 4 NCH_3) from 3.20 to 3.45 and 3.60 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 4.57 (dd, J=7 and 8 Hz, 1H, 3 α), 4.75 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.24 (dd, J=12 and 4 Hz, 1H: 4 α), 5.32 (broad d, J=6 Hz, 1H: 5 α), 5.89 (d, J=9 Hz, 1H: 6 α), 5.90 (broad q, J = 7.5 Hz, 1H: 1β), 6.53 (d, J=9 Hz, 1H: NH 2), 6.53

(d, J=8 Hz, 2H: 4ε), 7.03 (d, J=8 Hz, 2H: 4δ), from 7.10 to 7.35 (mt, 5H: aromatic H 6), 7.46 (mt, 2H: 1'H_5 and 1'H_4), 7.85 (dd, J=5.5 and 2 Hz, 1H: 1'H_6), 8.44 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6), 11.63 (s, 1H: OH).

4.2: Preparation of 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as described in Example 3, with 1 ml of a 5 g/l aqueous solution of (S)-4-aminophenylalanine being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9 and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 65% 100 mM phosphate buffer, pH 2.9, and 35% ac tonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethan . The organic phase is

washed with water, dried over sodium sulphate and then vaporated. 5 mg of 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A ar obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.72 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.90 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.10 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.19 (mt, 1H, 5 δ_2), 2.33 (broad d, J=16 Hz, 1H: 5 δ_1), 2.42 (d, J=16 Hz, 1H: 5 β_1), 2.81 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 2.90 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.24 (s, 3H: NCH_3 4), from 3.20 to 3.40 and 3.54 (2 mts, 1H each: CH_2 3 δ), 3.30 (t, J=12 Hz, 1H: 4 β_1), 3.72 (unres.comp., 2H: $ArNH_2$), 4.54 (dd, J=7.5 and 7 Hz, 1H, 3 α), 4.73 (broad dd, J=13 and 8 Hz, 1H: 5 ϵ_1), 4.82 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.22 (dd, J=12 and 4 Hz, 1H: 4 α), 5.32 (broad d, J=5.5 Hz, 1H: 5 α), 5.89 (mt, 2H: 6 α and 1β), 6.51 (d, J=9.5 Hz, 1H: NH 2) 6.61 (d J=8 Hz, 2H: 4ε), 6.98 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.45 (dd, J=8.5 and 1.5 Hz, 1H: 1'H_4), 7.48 (dd, J=8.5 and 4 Hz, 1H: 1'H_5), 7.82 (dd, J=4 and 1.5 Hz, 1H: 1'H_6), 8.43 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.63 (s, 1H: OH).

Example 5: Preparation of 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_A, of 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H and of 5-γ-hydroxy-4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-methylthiophenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 65 mg of dry residue are obtained. This is taken up in 6 ml of a mixture consisting of 60% water and 40% ac tonitrile and injected in two batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture

consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combin d and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 45 mg of 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.68 (dd, J=16 and 5.5 Hz, 1H 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3, 2 γ), 1.13 (mt, 1H: 3 β_2), from 1.25 to 1.40 (mt, 1H: 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.55 to 1.85 (mt, 3H: 3 γ_1, and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.18 (mt, 1H, 5 δ_2), 2.38 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.46 (s, 3H: SCH_3), 2.48 (d, J=16 Hz, 1H, 5 β_1), 2.85 (dt, J=13.5 and 4 Hz, 1H: 5 ϵ_2), 3.00 (dd, J=12 and 5 Hz, 1H: 4 β_2), 3.23 (s, 3H: NCH_3, 4), 3.37 (t, J=12 Hz, 1H: 4 β_1), 3.37 and 3.58 (2 mts, 1H each: CH_2 3 δ), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.77 (broad dd, J=13.5 and 8 Hz, 1H: 5 ϵ_1), 4.86 (mt, 1H: 2α), 4.89 (dd, J=10 and 1.5 Hz, 1H: 1α), 5.30 (broad d, J=5.5 Hz, 1H: 5 α), 5.32 (dd, J=12 and 5 Hz, 1H: 4 α), 5.90 (d, J=9.5 Hz, 1H: 6 α), 5.92 (dq, J=7.5 and 1.5 Hz, 1H: 1β), 6.55 (d, J=9.5 Hz, 1H: NH 2), 7.13 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.19 (d, J=8 Hz, 2H: 4ϵ), 7.45 (mt, 2H: 1'H_4 and H_5), 7.76 (t, J=5 Hz, 1'H_6), 8.42 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.65 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I_B, 10 mg of 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_B are isolated by means of semi-preparative column chromatography as described above but bringing the proportion of acetonitrile in the eluent phase to 50%.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.32 (mt, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.20 to 1.35 (mt, 2H: 3 β_2 and 3 γ_2), 1.30(d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.35 to 2.05 (mt, 9H: 3 γ_1 - 3 β_1 - CH_2 2 β - CH_2 5 δ - CH_2 5γ and 5 β_1), 2.44 (dt, J=13.5 and 1.5 Hz, 1H: 5 ϵ_2), 2.49 (s,3H: SCH_3), 2.99 (dd, J=12 and 5 Hz, 1H: 4 β_2), 3.09 (dd, J=12.5 and 12 Hz, 1H: 4 β_1), 3.54 and 3.64 (2 mts, 1H each: CH_2 3 δ), 4.17 (dd, J=7 and 6 Hz, 1H: 3 α), 4.49 (broad d, J=13.5 Hz: 1H: 5 ϵ_1), from 4.70 to 4.80 (mt, 3H: 2α - 5 α and 4 α), 4.84 (dd, J=10 and 1.5 Hz, 1H: 1α), 5.51 (d, J=7 Hz, 1H: 6 α), 5.73 (mt, 1H: 1β), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.10 (d, J=8 Hz, 2H: 4δ), 7.22 (d, J=8 Hz, 2H: 4ϵ), from 7.20 to 7.40 (mt, 7H: aromatic H 6 = 1' H_4 and 1'H_5), 7.87 (d, J=4 Hz, 1H: 1'H_6), 8.55 (unres.comp., 1H: NH 6), 8.55 (d, J=10 Hz, 1H: NH 1), 11.70 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I, 3 mg of 5γ-hydroxy-4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_B are

isolated by carrying out semi-preparative column chromatography as described above and maintaining the proportion of acetonitrile in the eluent phase at 45%.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): a markedly preponderant isomer is observed: the -OH in the 5 γ position in an axial position. 0.37 (d mt, J=16 Hz, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.20 to 1.45 (mt, 2H: 3 β_2 and 3 γ_2) 1.31 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.40 to 1.85 (mt, 5H: 3 γ_1 - CH_2 2 β and CH_2 5 δ), 1.98 (mt, 1H, 3 β_1), 2.17 (d, J=16 Hz, 1H: 5 β_1), 2.50 (s, 3H: SCH_3), 2.77 (dt, J=13.5 and 2 Hz, 1H: 5 ϵ_2), 2.99 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.11 (t, J=12 Hz, 1H: 4 β_1), from 3.45 to 3.70 (mt, 2H: CH_2 3 δ), 3.73 (mt, 1H: 5 γ in an equatorial position), 4.13 (t, J=7 Hz, 1H, 3 α), 4.37 (broad d, J=13.5 Hz, 1H: 5 ϵ_1), from 4,75 to 4.95 (mt, 3H: 2α, 4 α and 5 α), 4.89 (dd, J=10 and 1 Hz, 1H: 1α), 5.70 (d, J=8 Hz, 1H: 6 α), 5.80 (dq, J=7.5 and 1 Hz, 1H: 1β), 6.37 (d, J=5 Hz, 1H: NH 4), 6.71 (d, J=10 Hz, 1H: NH 2), 7.10 (d, J=8 Hz, 2H: 4δ), 7.22 (d, J=8 Hz, 2H: 4 ϵ), from 7.20 to 7.40 (mt, 5H: aromatic H 6), 7.43 (dd, J=8.5 and 1.5 Hz, 1H: 1'H_4), 7.46 (dd, J=8.5 and 4 Hz, 1H: 1'H_5), 7.89 (dd, J=4 and 1.5 Hz, 1H: 1'H_6), 8.55 (d, J=10 Hz, 1H: NH 1), 9.15 (d, J=8 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 6: Preparation of 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A and of 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_B

Strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-methylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 49 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected, in two batches, onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphat buffer, pH 2.9, and 45% acetonitrile. The fractions containing

the new pristinamycin ar combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried ov r sodium sulphate and then evaporated. 44 mg of 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.52 (dd, J=16 and 6 Hz, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.15 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.04 (mt, 1H, 3 β_1), 2.18 (mt, 1H, 5 δ_2), from 2.25 to 2.45 (mt, 2H: 5 δ_1 and 5 β_1), 2.36 (s, 3H: $ArCH_3$), 2.83 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 2.99 (dd, J=13 and 4 Hz, 1H: 4 β_2), 3.28 (s, 3H: $NCH_3$4), 3.31 and 3,59 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=13 Hz, 1H: 4 β_1), 4.59 (t, J=7.5 Hz, 1H, 3 α), 4.74 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.85 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), from 5.25 to 5.35 (mt, 2H: 5 α and 4 α), from 5.85 to 5.95 (mt, 2H: 6 α and 1β), 6.52 (d, J=9.5 Hz, 1H: NH 2), 7.14 (AB limit, J=9 Hz, 4H: 4δ and 4ϵ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.50 (mt, 2H: 1'H_4 and 1'H_5), 7.81 (dd, J=4 and 2Hz, 1H: 1'H_6), 8.41 (d, J=10 Hz, 1H: NH 1), 8.74 (d, J=9 Hz, 1H: NH 6), 11.63 (s, 1H:OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 21 mg of 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_H (mass spectrometry:

$M+H^+=810$) are isolated by carrying out semi-preparative column chromatography as d scribed above.

EXAMPLE 7: Pr paration of 4ζ-methoxy-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 12 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (RS)-4-methoxyphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 0.35 litres of must recovered from the 12 Erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 14 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Machery Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and

40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with on volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 12 mg of 4ζ-methoxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, d in ppm, ref. TMS): 0.63 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.96 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.17 (mt, 1H: 3 β_2), from 1.30 to 1.45 (mt, 1H: 3 γ_2), 1.38 (d, J=7.5 Hz, 3H: CH_3 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.05 (mt, 1H, 3 β_1), 2.20 (mt, 1H, 5 δ_2), 2.40 (broad d, J=16 Hz, 1H: 5 δ_1), 2.47 (d, J=16 Hz, 1H: 5 β_1), 2.88 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 2.99 (dd, J=12.5 and 5 Hz, 1H: 4 β_2), 3.30 (s, 3H: NCH_3 4), 3.32 and 3.60 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12.5 Hz, 1H: 4 β_1), 3.80 (s, 3H: OCH_3), 4.60 (t, J=7.5 Hz, 1H, 3 α), 4.80 (broad dd, J=13 and 8.5 Hz, 1H: 5 ϵ_1), 4.88 (mt, 1H: 2α), 4.92 (broad d, J=10 Hz, 1H: 1α), 5.31 (dd, J=12.5 and 5 Hz, 1H: 4 α), 5.34 (broad d, J=5.5 Hz, 1H: 5 α), 5.90 (d, J=9 Hz, 1H: 6 α), 5.93 (broad q, J=7.5 Hz, 1H: 1β), 6.54 (d, J=9 Hz, 1H: NH 2), 6.87 (d, J=8 Hz, 2H: 4ϵ), 7.16 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.40 (mt, 5H: aromatic H 6), 7.50 (mt, 2H: 1'H_5 and 1'H_4), 7.80 (dd, J=4 and 2.5 Hz, 1H: 1'H_6), 8.43 (d, J=10 Hz, 1H: NH 1), 8.78 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

EXAMPLE 8: Pr paration of 4ζ-methoxycarbonyl-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-methoxycarbonylphenylalanine, synthesized as in Example 33, being added at 16 h. At the end of 24 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 14 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one

volume of dichloromethane. The organic phase is washed with water, dri d over sodium sulphate and then evaporated. 9 mg of 4ζ-methoxycarbonyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.70 (dd, J=16 and 6 Hz, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.08 (mt, 1H: 3 β_2), from 1.30 to 1.40 (mt, 1H: 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.40 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.48 (d, J=16 Hz, 1H, 5 β_1), 2.89 (dt, J=14.5 and 4.5 Hz, 1H: 5 ϵ_2), 3.10 (dd, J=13.5 and 6 Hz, 1H: 4 β_2), 3.24 (s, 3H: NCH_3 4), 3.38 and 3.61 (2 mts, 1H each: CH_2 3 δ), 3.47 (t, J=13.5 Hz, 1H: 4 β_1), 3.96 (s, 3H: $COOCH_3$), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.78 (broad dd, J=14.5 and 8 Hz, 1H: 5 ϵ_1), 4.86 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.33 (broad d, J=6 Hz, 1H: 5 α), 5.42 (dd, J=13.5 and 6 Hz, 1H: 4 α), 5.92 (d, (J=9.5 Hz) and mt, 1H each: 6 α and 1β respectively), 6.52 (d, J=10 Hz, 1H: NH 2), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.28 (d, J=8 Hz, 2H: 4δ), 7.43 (dd, J=9 and 1.5 Hz, 1H: 1'H_4), 7.47 (dd, J=9 and 5 Hz, 1H: 1'H_5), 7.66 (d, J=5 and 1.5 Hz, 1H: 1'H_6), 7.98 (d, J=8 Hz, 2H: 4ε), 8.38 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 9: Preparation of 4ζ-chloro-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-chlorophenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is

washed with water, dried ov r sodium sulphate and then evaporated. 1 mg of 4ζ-chloro-de(4ζ-dimethylamino)pristinamycin I_A is obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 0.95 (dd, J=16 and 5 Hz, 1H, 5 β_2), 1.09 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ) from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.17 (mt, 1H, 5 δ_2), 2.43 (broad d, J=16 Hz, 1H: 5 δ_1), 2.59 (d, J=16 Hz, 1H: 5 β_1), 2.90 (dt, J=13.5 and 4 Hz, 1H: 5 ϵ_2), 3.04 (dd, J=13 and 6 Hz, 1H: 4 β_2), 3.21 (s, 3H: 4 NCH_3), 3.36 (t, J=13 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH_2 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13.5 and 8 Hz, 1H: 5 ϵ_1), 4.86 (mt, 1H: 2α), 4.87 (broad d, J=10 Hz, 1H: 1α), 5.38 (mt, 2H: 5 α and 4 α), 5.93 (mt, 2H: 6 α and 1β), 6.52 (d, J=10 Hz, 1H: NH 2), 7.12 (d, J=8 Hz, 2H: 4δ) from 7.15 to 7.35 (mt, 7H: aromatic H 6 and 4ε), 7.38 (dd, J=9 and 4.5 Hz, 1H:1'H_5), 7.43 (broad d, J=9 Hz, 1H: 1'H_4), 7.68 (dd, J=4.5 and 1 Hz, 1H: 1'H_6), 8.36 (d, J=10 Hz, 1H: NH 1), 8.75 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

EXAMPLE 10: Preparation of 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_A and of 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_B.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution

of (R,S)-4-bromophenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the nd of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 6 mg of 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H: CH_3 2 γ), 0.95 (dd, J=16

and 5 Hz, 1H, 5 β_2), 1.10 (mt, 1H: 3 β_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ) 1.36 (mt, 1H: 3 γ_2), from 1.50 to 1.85 (mt, 3H, 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.18 (mt, 1H: 5 δ_2), 2.43 (broad d, J=16 Hz, 1H: 5 δ_1), 2.59 (d, J=16 Hz, 1H: 5 β_1), 2.90 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4 β_2), 3.21 (s, 3H: 4 NCH_3), 3.33 (dd, J=13-11 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH_2 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.86 (mt, 1H, 2α), 4.89 (d broad, J=10 Hz, 1H: 1α), 5.37 (broad d, J=5 Hz, 1H: 5 α), (dd, J=11 and 5.5 Hz, 1H: 4 α), 5.92 (mt, 2H: 6 α and 1β), 6.56 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.40 (mt, 4H: 1'H_4 - 1'H_5 and 4ϵ), 7.70 (broad d, J=5 Hz, 1H: 1'H_6), 8.40 (d, J=10 Hz, 1H: NH 1), 8.77 (d, J=9 Hz, 1H: NH 6), 11.68 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 3 mg of 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_H (mass spectrometry: $M+H^+$=874) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 11: Preparation of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_A and of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_H.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution

of (RS)-4-iodophenylalanine in sodium hydroxide solution b ing add d at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 12 mg of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H: CH_3 2 γ), 0.95 (dd, J=16

and 5.5 Hz, 1H: 5 β_2), 1.10 (mt, 1H: 3 β_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), 1.38 (mt, 1H: 3 γ_2), from 1.55 to 1.85 (mt, 3H, 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.17 (mt, 1H: 5 δ_2), 2.43 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.60 (d, J=16 Hz, 1H: 5 β_1), 2.89 (dt, J=14 and 4.5 Hz, 1H: 5 ϵ_2), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4 β_2), 3.21 (s, 3H: NCH_3 4), 3.31 (dd, J=13 and 11 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH_2 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.75 (broad dd, J=14 and 8 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2α), 4.88 (broad d, J=10 Hz, 1H: 1α), 5.37 (broad d, J=5.5 Hz, 1H: 5 α), 5.39 (dd, J=11 and 5.5 Hz, 1H: 4 α), 5.92 (mt, 2H: 6 α and 1β), 6.54 (d, J=9.5 Hz, 1H: NH 2), 6.94 (d, J=7.5 Hz, 2H: 4δ), from 7.15 to 7.50 (mt, 5H: aromatic H 6), 7.36 (dd, J=9 and 4 Hz, 1H: 1'H_5), 7.43 (broad d, J=9 Hz, 1H: 1'H_4), 7.62 (d, J=7.5 Hz, 2H: 4ϵ), 7.68 (d, J=4 Hz, 1H: 1'H_6), 8.38 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 6 mg of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_H (mass spectrometry: $M+H^+=922$) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 12 Preparation of 4ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_A and of 4ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_H.

Strain SP92::pVRC508 is cultured in

production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (S)-4-trifluoromethylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 5 mg of 4ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR sp ctrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.86 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2γ), 1.13 (mt, 1H: 3 β_2), 1.31 (d, J=7.5 Hz, 3H: CH_3 1 γ) 1.42 (mt, 1H: 3 γ_2), from 1.55 to 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.15 (mt, 1H, 5 δ_2), 2.40 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.55 (d, J=16 Hz, 1H: 5 β_1), 2.88 (dt, J=14 and 4 Hz, 1H: 5 ϵ_2), 3.18 (s, 3H: NCH_3 4), 3.20 and 3.31 (2 dd, respectively J=13 and 6 Hz and J=13 and 10 Hz, 1H each: 4 β_2 and 4 β_1), 3.42 and 3.60 (2 mts, 1H each: CH_2 3 δ), 4.50 (t, J=7.5 Hz, 1H, 3 α), 4.73 (broad dd, J=14 and 7.5 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2α), 4.91 (broad d, J=10 Hz, 1H: 1α), 5.40 (broad d, J=5.5 Hz, 1H: 5 α), 5.55 (dd, J=10 and 6 Hz, 1H: 4 α), 5.87 (d, J=9.5 Hz, 1H: 6 α), 5.90 (broad q, J=7.5 Hz, 1H: 1β), 6.68 (d, J=9.5 Hz, 1H: NH 2), from 7.15 to 7.40 (mt, 9H: 4δ-aromatic H 6 - 1'H_5 and 1'H_4), 7.52 (d, J=8 Hz, 2H: 4ϵ), 7.68 (d, J=4 and 1.5 Hz, 1H: 1'H_6), 8.43 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_B, 4 mg of ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_B (mass spectrometry: $M+H^+$=864) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 13: Preparation of 4ζ-tert-butyl-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-tert-butylphenylalanine, synthesized as in Example 35-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyers are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with

on volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 30 mg of 4ζ-tert-butyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS, ref. TMS): 0.21 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.17 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.33 (s, 9H: CH_3 of tert-butyl), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.04 (mt, 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.30 (mt, 2H: 5 δ_1 and 5 β_1), 2.80 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 3.00 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.29 (s, 3H: $NCH_3$4), 3.31 and 3.59 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 4.57 (t, J=7.5 Hz, 1H, 3 α), 4.74 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.85 (mt, 1H: 2α), 4.90 (broad d, J=10 Hz, 1H: 1α), 5.21 (broad d, J=5.5 Hz, 1H: 5 α), 5.25 (dd, J=12 and 4 Hz, 1H: 4 α), 5.87(d, J=9 Hz, 1H: 6 α), 5.92 (broad q, J=7.5 Hz, 1H: 1 [lacuna] 1H: 1'H_6), 8.45 (d, J=10 Hz, 1H: NH 1), 8.74 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

EXAMPLE 14: Preparation of 4ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_A and of 4ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_H.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-isopropylphenylalanine, synthesized as in Example 36-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 61 mg of the dry residue are obtained. This residue is taken up in 9 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 3 batches onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer,

pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 51 mg of 4ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (250 MHz, $CDCl_3$, δ in ppm, ref. TMS, ref. TMS): 0.31 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.00 to 1.45 (mt, 2H: 3 β_2 and 3 γ_2), 1.25 (d, J=7.5 Hz, 6H: CH_3 of isopropyl), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), from 1.95 to 2.20 (mt, 2H, 3 β_1 and 5 δ_2), 2.30 (mt, 2H: 5 δ_1 and 5 β_1), 2.80 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 2.88 (mt, 1H: CH of isopropyl), 2.98 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.30 (s, 3H: NCH_3 4), 3.32 and 3.55 (2 mts, 1H each: CH_2 3 δ), 3.38 (t, J=12 Hz, 1H: 4 β_1), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.72 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.85 (mt, 1H: 2α), 4.88 (broad d, J=10 Hz, 1H: 1α), 5.21 (broad d, J=5.5 Hz, 1H: 5α), 5.25 (dd, J=12 and 4 Hz, 1H: 4 α), 5.87 (d, J=9 Hz, 1H: 6 α), 5.90 (broad q, J=7.5 Hz, 1H: 1β), 6.50 (d, J=9.5 Hz, 1H: NH 2), from 7.05 to 7.35 (mt, 9H: aromatic H 6 - 4ϵ and 4δ), 7.50 (mt, 2H: 1'H_5 and 1'H_4), 7.86 (dd, J=4 and 1.5 Hz, 1H: 1'H_6), 8.40 (d, J=10 Hz, 1H: NH 1), 8.72 (d, J=9 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

Using the same fractions derived from the silica column described above, which fractions also

contain the new derivative of pristinamycin I_E, 5 mg of ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_E are isolated by carrying out semi-preparative column chromatography as described above.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.20 (mt, 1H, 5 β_2), 0.92 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.15 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.24 (d, J=7.5 Hz, 6H: CH_3 of isopropyl), 1.34 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.35 to 2.05 (mt, 9H: 3 γ_1 - 3 β_1 - CH_2 2 β - CH_2 5 δ - CH_2 5 γ and 5 β_1), 2.45 (dt, J=13 and 1.5 Hz, 1H: 5ϵ_2), 2.89 (mt, 1H: ArCH), 3.09 (dd, J=14 and 7 Hz, 1H: 4 β_2), 3.17 (s, 3H: NCH_3 4), 3.25 (dd, J=14 and 9 Hz, 1H: 4 β_1), 3.32 and 3.52 (2 mts, 1H each: CH_2 3 δ), 4.55 (mt, 2H: 3 α and 5 ϵ_1), 4.80 (mt, 1H: 2α), 4.89 (dd, J=10 and 1.5 Hz, 1H: 1α), 4.90 (mt, 1H: 5 α), 5.35 (dd, J=9 and 7 Hz, 1H: 4 α), 5.60 (d, J=8 Hz, 1H: 6 α), 5.89 (dq, J=7.5 and 1.5 Hz, 1H: 1β), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4δ), 7.14 (d, J=8 Hz, 2H: 4ε), from 7.20 to 7.40 (mt, 7H: aromatic H 6 - 1'H_4 and 1'H_5), 7.77 (broad d, J=4 Hz, 1H: 1'H_6), 8.46 (d, J=10 Hz, 1H: NH 1), 8.48 (d, J=8 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 15: Preparation of 4ε-methylamino-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-methylaminophenylalanine, synthesized as in

Example 35-3, in water b ing added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% of 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 19 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 8 mg of 4ϵ-methylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm,

ref. TMS): 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.00 (dd, J=16 and 6 Hz, 1H, 5 β_2), 1.17 (mt, 1H: 3 β_2), from 1.25 to 1.40 (mt, 2H: 3 γ_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.55 to 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.03 (mt, 1H, 3 β_1), 2.23 (mt, 1H, 5 δ_2), 2.39 (broad d, J=16 Hz, 1H: 5 δ_1), 2.52 (d, J=16 Hz, 1H: 5 β_1), 2.78 (s, 3H: $ArNCH_3$ 4), 2.85 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 2.99 (dd, J=13 and 4.5 Hz, 1H: 4 β_2), 3.23 (s, 3H: NCH_3 4), 3.25 (t, J=13 Hz, 1H: 4β_1), 3.38 and 3.58 (2 mts, 1H each: CH_2 3 δ), 4.05 (unres. comp., 1H: ArNH), 4.58 (dd, J=6.5 and 7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13 and 8 Hz, 1H: 5 ϵ_1), 4.85 (mt, 1H: 2α), 4.87 (broad d, J=10 Hz, 1H: 1α), 5.35 (dd, J=13 and 4.5 Hz, 1H: 4 α), 5.38 (broad d, J=6 Hz, 1H: 5 α), 5.90 (d, J=9.5 Hz, 1H: 6 α), 5.91 (mt, 1H: 1β), 6.36 (broad s, 1H: H 2 of the aromatic moiety at position 4), from 6.45 to 6.55 (mt, 2H: H4 and H6 of the aromatic moiety in position 4), 6.53 (d, J=10 Hz, 1H: NH 2), 7.12 (t, J=8 Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.15 to 7.45 (mt, 5H: aromatic H 6), 7.35 (mt, 2H: 1′ H_4 and 1′ H_5), 7.75 (t, J=3 Hz, 1H: 1′ H_6), 8.40 (d, J=10 Hz, 1H: NH 1), 8.78 (d, J=9.5 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

EXAMPLE 16: Preparation of 4ϵ-methoxy-de(4ζ-dimethylamino)pristinamycin I_A and of 4ϵ-methoxy-de(4ζ-dimethylamino)pristinamycin I_H.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution

of (S)-3-methoxyphenylalanine in 0.1N sodium hydroxide solution being added at 16 h. At th end of 40 h of cultur , the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 41 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7μ C8 10×250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 28 mg of 4ϵ-methoxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm,

ref. TMS): 0.52 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.90 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.10 to 1.34 (mt, 2H: 3 β_2 and 3 γ_2), 1.34 (d, J=7.5 Hz, 3H: CH_3 1γ), from 1.50 to 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.40 (mt, 1H, 3 β_1), 2.20 (mt, 1H, 5 δ_2), 2.35 (broad d, J=16 Hz, 1H: 5 δ_1), 2.38 (d, J=16 Hz, 1H: 5 β_1), 2.83 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 2.97 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.28 (s, 3H: NCH_3 4), 3.28 and 3.56 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 3.80 (s, 3H: OCH_3), 4.58 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13 and 8 Hz, 1H: 5 ϵ_1), 4.85 (mt, 1H: 2α), 4.90 (broad d, J=10 Hz, 1H: 1α): 5.27 (dd, J=12 and 4 Hz, 1H: 4 α), 5.30 (broad d, J=5.5 Hz, 1H: 5 α), 5.89 (d, J=9.5 Hz, 1H: 6 α), 5.91 (broad q, J=7.5 Hz, 1H: 1β), 6.51 (d, J=10 Hz, 1H: NH 2), from 6.80 to 6.90 (mt, 3H: H 2 - H 4 and H 6 of the aromatic moiety in position 4), from 7.15 to 7.40 (mt, 6H: H 5 of the aromatic moiety in position 4 and aromatic H 6), 7.45 (broad d, J=9 Hz, 1H: 1'H_4), 7.50 (dd, J=9 and 4 Hz, 1H:1'H_5), 7.80 (broad d, J = 4 Hz, 1H: 1'H_6), 8.40 (d, J=10 Hz, 1H: NH 1), 8.73 (d, J=9.5 Hz, 1H: NH 6), 11.62 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_B, 7 mg of 4ϵ-methoxy-de(4ζ-dimethylamino)pristinamycin I_B (mass spectrometry: $M+H^+$ = 826) are isolated by carrying out semi-preparativ column chromatography as described above.

EXAMPLE 17: Preparation of 4ε-fluoro-4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-fluoro-4-methylphenylalanine, synthesized as in Example 34-5, in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 15 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new

pristinamycin ar combin d and extracted with one volume of dichlorom thane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 9 mg of 4ε-fluoro-4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.60 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.12 (mt, 1H: 3 β_2), from 1.25 to 1.35 (mt, 1H: 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.27 (s, 3H: $ArCH_3$), 2.36 (broad d, J=16 Hz, 1H: 5 δ_1), 2.45 (d, J=16 Hz, 1H: 5 β_1), 2,85 (dt, J=13 and 4.5 Hz, 1H: 5 ϵ_2), 2.97 (dd, J=12.5 and 4.5 Hz, 1H: 4 β_2), 3.23 (s, 3H: NCH_3 4), 3.30 and 3.56 (2 mts, 1H each: CH_2 3 δ), 3.37 (t, J=12.5 Hz, 1H: 4 β_1), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.75 (broad dd, J=13 and 8 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.29 (dd, J=12.5 and 4.5 Hz, 1H: 4 α), 5.32 (broad d, J=5.5 Hz, 1H: 5 α), 5.89 (d J=9.5 Hz, 1H: 6 α), 5.92 (mt, 1H: 1β), 6.49 (d, J=10 Hz, 1H: NH 2), 6.90 (mt, 2H: H 2 and H 6 of the aromatic moiety in position 4), 7.11 (t, J=8 Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.10 to 7.30 (mt, 5H: aromatic H 6), 7.43 (dd, J=8.5 and 1 Hz, 1H: 1'H_4), 7.49 (dd, J=8.5 and 4.5 Hz, 1H: 1'H_5), 7.75 (dd, J=4.5 and 1 Hz, 1H: 1'H_6), 8.48 (d, J=10 Hz, 1H: NH 1), 8.70 (d, J=9.5 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

EXAMPLE 18: Preparation of 4ζ-ethylamino-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50 erlenmeyer flasks are extracted with 2 volumes of a mixture of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% ac tonitrile. The fractions containing 4ζ-ethylamino-de(4ζ-

dimethylamino)pristinamycin I_A are combin d and extracted with one volume of dichloromethane. The organic phas is wash d with water, dri d over sodium sulphate and then evaporated. 10 mg of 4ζ-ethylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.72 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.15 (mt, 1H: 1H of the CH_2 in 3 β); from 1.20 to 1.40 (mt, 1H: 1H of the CH_2 in 3 γ); 1.27 (t, J = 7.5 Hz, 3H: CH_3 of the ethyl); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.50 to 1.65 (mt, 1H: the other H of the CH_2 in 3 γ); 1.60 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of CH_2 in 3 β); 2.21 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.40 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.82 (dt, J = 13 and 4.5 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.89 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.10 (mt, 2H: NCH_2 of the ethyl); from 3.20 to 3.35 (mt, 1H: 1H of the CH_2 in 3 δ); 3.26 (s, 3H: NCH_3); 3.31 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.54 (mt, 1H: the other H of the CH_2 in 3 δ); 3.67 (unres. comp., 1H: NH); 4.56 (dd, J = 6.5 and 7 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.90 (broad d, J = 10 Hz, 1H : 1 α) ; 5.24 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 6 Hz, 1H: 5 α); 5.88 (d, J = 9.5 Hz, 1H : 6 α); 5.90 (mt, 1H : 1 β); 6.52 (d, J = 8 Hz, 3H : NH in 2 and

aromatic H in 4 ε); 7.00 (d, J = 8 Hz, 2H : aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.46 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.84 (dd, J = 4 and 1 Hz, 1H: $1'H_6$); 8.45 (d, J = 10 Hz, 1H: NH in 1); 8.77 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

EXAMPLE 19: Preparation of 4ζ-diethylamino-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-diethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50 erlenmeyer flasks are extracted with 2 volumes of a mixture of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-diethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and vaporated. The dry residue is taken up in

7 ml of a mixture consisting of 60% water and 40% acetonitrile and inject d in two portions onto a semi-preparativ Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 68% 100 mM phosphate buffer, pH 2.9, and 32% acetonitrile. The fractions containing 4ζ-diethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 50 mg of 4ζ-diethylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.65 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.14 (t, J = 7 Hz, 6H: CH_3 of the ethyl); 1.15 (mt, 1H: 1H of the CH_2 in 3 β); 1.26 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.55 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β): 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.22 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.37(d, J=16 Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, J = 13 and 4.5 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.89 (dd, J = 12.5 and 4 Hz, 1H : 1H of the CH_2 in 4 β); from 3.20 to 3.40 (mt, 6H: NCH_2 of the ethyl - 1H of the CH_2 in 3 δ and the other H of the CH_2 in 4 β); 3.27 (s, 3H: NCH_3); 3.55 (mt, 1H: the other H of the CH_2 in 3 δ); 4.58 (dd, J = 8 and 6 Hz, 1H: 3 α); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the

other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α) ; 5.21 (dd, J = 12.5 and 4 Hz, 1H: 4 α); 5.28 (broad d, J = 6 Hz, 1H : 5 α); 5.87 (d, J = 9.5 Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.52 (d, J = 9.5 Hz, 1H : NH in 2); 6.60 (d, J = 8 Hz, 2H: aromatic H in 4 ϵ); 7.02 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.46 (limiting AB, 2H: 1'H_4 and 1'H_5); 7.88 (dd, J = 4.5 and 2.5 Hz, 1H: 1'H_6); 8.43 (d, J = 10 Hz, 1H: NH in 1); 8.76 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 20: Preparation of 4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 94 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-diallylaminophenylalanine dihydrochloride, synthesized as in Example 38-1, in water being added at 16h. At the end of 40h of culture, the 2.8 litres of must recovered from the 94 erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is

mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichlorom thane. The fractions containing 4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Machery Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% acetonitrile. The fractions containing 4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 15 mg of 4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref.TMS): 0.55 (dd, J = 16 and 6 Hz, 1H : 1H of the CH_2 in 5 β); 0.93 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.18 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H : 1H of the CH_2 in 3 γ); 1.34 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.59 (mt, 1H: the other H of the CH_2 in 3 γ); 1.68 and 1.78 (2 mts, 1H each: CH_2 in 2 β); 2.04 (mt, 1H: the other H of CH_2 in 3 β); 2.25 and 2.34 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.40 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, J = 13 and 4.5 Hz, 1H: 1H of the CH_2 in 5 ε); 2.92 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.20 to 3.30

(mt, 1H: 1H of the CH_2 in 3 δ); 3.29 (s, 3H: NCH_3); 3.33 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 3.93 (limiting AB, 4H: NCH_2 of the allyl); 4.60 (dd, J = 8 and 6.5 Hz, 1H: 3 α); 4.78 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.87 (mt, 1H: 2 α); 4.92 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.10 to 5.25 (mt, 5H: 4 α and $=CH_2$ of the allyl); 5.28 (broad d, J = 6 Hz, 1H: 5 α); 5.85 (mt, 2H: CH= of the allyl); 5.92 (d, J = 9.5 Hz, 1H: 6 α); 5.94 (mt, 1H : 1 β); 6.54 (d, J = 10 Hz, 1H: NH in 2); 6.65 (d, J = 8 Hz, 2H : aromatic H in 4 ϵ); 7.05 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.51 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.88 (dd, J = 4 and 2 Hz, 1H: $1'H_6$); 8.43 (d, J = 10 Hz, 1H: NH in 1); 8.77 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H : OH).

EXAMPLE 21: Preparation of 4ζ-allylethylamino-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 26 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-allylethylaminophenylalanine dihydrochloride, synthesized as in Example 39-4, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 0.78 litre of must recovered from the 26 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM

phosphate buffer, pH 2.9, and 34% acetonitril , and then centrifuged. The supernatant is extracted with 2 tim s 0.5 volumes of dichlorom thane. Th chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-allylethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-allylethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 20 mg of 4ζ-allylethylamino-de(4ζ-dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.58 (dd, J = 16 and 6 Hz, 1H: 1H of CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.16 (t, J = 7 Hz, 3H: CH_3 of the ethyl); 1.16 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H: 1H of CH_2 in 3 γ); 1.32

(d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.54 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.23 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.37 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, J = 13 and 4.5 Hz, 1H : 1H of CH_2 in 5 ϵ); 2.87 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.15 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.26 (s, 3H: NCH_3); 3.30 (t, J = 12 Hz, 1H: the other H of CH_2 in 4 β); 3.36 (mt, 2H: NCH_2 of the ethyl); 3.54 (mt, 1H: the other H of the CH_2 in 3 δ); 3.90 (limiting AB, 2H: NCH_2 of the allyl); 4.57 (dd, J = 8 and 6 Hz, 1H: 3 α); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.05 to 5.20 (mt, 3H: 4 α and $=CH_2$ of the allyl); 5.27 (broad d, J = 6 Hz, 1H : 5 α); 5.83 (mt, 1H: CH= of the allyl); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.50 (d, J = 10 Hz, 1H: NH in 2); 6.60 (d, J = 8 Hz, 2H: aromatic H in 4 ϵ); 7.02 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.47 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.88 (dd, J = 4 and 2 Hz, 1H: $1'H_6$); 8.41 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 22: Preparation of the 4ζ-ethylpropylamino-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylpropylaminophenylalanine dihydrochloride, synthesized as in Example 39-6, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litre of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethylpropylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 63% 100 mM phosphate buffer, pH 2.9, and 37% of acetonitrile. The fractions containing

4ζ-ethylpropylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 16 mg of 4ζ-ethylpropylamino-de(4ζ-dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.67 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 0.95 (t, J = 7.5 Hz, 3H: CH_3 of propyl); 1.14 (t, J = 7 Hz, 3H : CH_3 of the ethyl); 1.15 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H : CH_3 in 1 γ); from 1.45 to 1.65 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 propyl); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.23 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.37 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, J = 13 and 5 Hz, 1H: 1H of the CH_2 in 5 ε); 2.89 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.10 to 3.25 (mt, 3H: 1H of the CH_2 in 3 δ and NCH_2 of the propyl); 3.26 (s, 3H: NCH_3); from 3.25 to 3.40 (mt, 2H: NCH_2 of the ethyl); 3.34 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.54 (mt, 1H: the other H of the CH_2 in 3 δ); 4.57 (dd, J = 7.5 and 6 Hz, 1H: 3 α); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H : 1 α); 5.21 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.28 (broad

d, J = 6 Hz, 1H: 5 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.48 (d, J = 10 Hz, 1H: NH in 2); 6.60 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.03 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.47 (limiting AB, 2H: 1'H_4 and 1'H_5); 7.89 (mt, 1H: 1'H_6); 8.42 (d, J = 10 Hz, 1H : NH in 1); 8.76 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 23: Preparation of the 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-O-trifluoromethyltyrosine hydrochloride, synthesized as in Example 34-8, in water being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times [lacuna] volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in [lacuna] ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% m thanol in

dichloromethane. The fractions containing 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residu is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected in two portions onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 46.5 mg of 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.77 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.08 (mt, 1H: 1H of the CH_2 in 3 β); from 1.30 to 1.40 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.55 to 1.70 (mt, 1H: the other H of the CH_2 in 3 γ); 1.65 and 1.76 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.11 and 2.40 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.54 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.88 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 3.08 (dd, J = 12 and 5 Hz, 1H: 1H of the CH_2 in 4 β); 3.22 (s, 3H: NCH_3); from 3.30 to 3.45

(mt, 1H: 1H of the CH_2 in 3 δ); 3.39 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.59 (mt, 1H: the other H of the CH_2 in 3 δ); 4.53 (t, J = 7.5 Hz, 1H : 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1.5 Hz, 1H: 1 α); 5.35 (broad d, J = 5.5 Hz, 1H: 5 α); 5.41 (dd, J = 12 and 5 Hz, 1H: 4 α); 5.92 (d, J = 10 Hz, 1H : 6 α); 5.93 (mt, 1H: 1 β); 6.53 (d, J = 9.5 Hz, 1H: NH in 2); from 7.15 to 7.35 (mt, 5H: aromatic H in 6); 7.16 (d, J = 8 Hz, 2H: aromatic H in 4 ϵ); 7.26 (d, J = 8 Hz, 2H: aromatic H in 4 δ); 7.37 (dd, J = 8.5 and 4 Hz, 1H: $1'H_5$); 7.42 (dd, J = 8.5 and 1.5 Hz, 1H: $1'H_4$); 7.70 (dd, J = 4 and 1.5 Hz, 1H: $1'H_6$); 8.37 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH in 6); 11.66 (s, 1H: OH).

EXAMPLE 24: Preparation of 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 90 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-allyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with

2 times 0.5 volumes of dichloromethane. The chlorom thylen phases ar washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS):0.63 (dd, J = 16 and 6 Hz, 1H: 1H of CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of CH_2 in 3 β); 1.29 (mt, 1H: 1H of CH_2 in 3 γ); 1.33 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.57 (mt, 1H: the other H of th CH_2 in 3 γ); 1.65 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β);

2.14 and 2.34 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.43 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.85 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.25 (s, 3H: NCH_3); 3.33 (mt, 1H: 1H of the CH_2 in 3 δ): 3.36 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.56 (mt, 1H: the other H of the CH_2 in 3 δ); 4.51 (limiting AB, 2H: OCH_2 of the allyl); 4.56 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.88 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.27 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 6 Hz, 1H: 5 α); 5.30 and 5.40 (respectively, mt and dd, J = 17 and 1.5 Hz, 1H each: $=CH_2$ of the allyl); 5.89 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.02 (mt, 1H: CH= of the allyl); 6.50 (d, J = 10 Hz, 1H: NH in 2); 6.85 (d, J = 8 Hz, 2H: aromatic H in 4 ϵ); 7.12 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.45 (dd, J = 8.5 and 1.5 Hz, 1H: $1'H_4$); 7.57 (dd, J = 8.5 and 4 Hz, 1H: $1'H_5$); 7.77 (dd, J = 4 and 1.5 Hz, 1H: $1'H_6$); 8.41 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 9.5 Hz, 1H: NH in 6); 11.63 (s, 1H: OH).

EXAMPLE 25: Preparation of 4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 90 erlenmeyer flasks, as

described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-ethyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethoxy-de(4ζ-dimethyl-amino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.64 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.12 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); 1.42 (t, J = 7 Hz, 3H: CH_3 of the ethyl); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.16 and 2.35 (respectively mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.43 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.93 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.15 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.24 (s, 3H: NCH_3); 3.35 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.55 (mt, 1H: the other H of the CH_2 in 3 δ); 3.95 (limiting AB, 2H: OCH_2 of the ethyl); 4.56 (dd, J = 7.5 and 6 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.87 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.26 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 5.5 Hz, 1H: 5 α); 5.88 (d, J = 10 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.48 (d, J = 10 Hz, 1H: NH in 2); 6.83 (d, J = 8 Hz, 2H: aromatic H in 4 ϵ); 7.10 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.44 (dd, J = 8.5 and 1.5 Hz, 1H: 1'H_4); 7.57 (dd, J = 8.5 and 4.5 Hz, 1H: 1'H_5); 7.77 (dd, J = 4.5 and 1.5 Hz, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H :

NH in 6); 11.60 (s, 1H: OH).

EXAMPLE 26: Preparation of 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride, synthesized as in Example 42-1, in water being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH

2.9, and 40% of acetonitril . Th fractions containing 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 3.2 mg of 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.66 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of the CH_2 in 3 β); 1.28 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.66 and 1.76 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.16 and 2.37 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ): 2.47 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.86 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.23 (s, 3H: NCH_3); 3.32 (mt, 1H: 1H of the CH_2 in 3 δ); 3.37 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 3.82 (t, J = 6 Hz, 2H: CH_2Cl); 4.19 (limiting AB, 2H: OCH_2 of the ethyl); 4.55 (dd, J = 7.5 and 7 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.87 (broad d, J = 10 Hz, 1H: 1 α); 5.28 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 5.5 Hz, 1H : 5 α); 5.88 (d, J = 10 Hz, 1H: 6α); 5.90 (mt, 1H :

1 β); 6.50 (d, J = 10 Hz, 1H: NH in 2); 6.86 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.13 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.45 (limiting AB, 2H: 1'H_4 and 1'H_5); 7.75 (dd, J = 4 and 2 Hz, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 10 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 27: Preparation of 4ζ-acetyl-de 4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-acetylphenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-acetyl)-d (4ζ-dimethyl-

amino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixtur consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4ζ-acetyl-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 4.2 mg of 4ζ-acetyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.73 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.93 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.12 (mt, 1H: 1H of the CH_2 in 3 β); from 1.25 to 1.45 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); 1.62 (mt, 1H: the other H of the CH_2 in 3 γ); from 1.60 to 1.85 (mt, 2H: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.20 and 2.42 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.52 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.60 (s, 3H: $ArCOCH_3$); 2.88 (dt, J = 13 and 4.5 Hz, 1H: 1H of CH_2 in 5 ϵ); 3.13 (dd, J = 13.5 and 5.5 Hz, 1H: 1H of the CH_2 in 4 β); 3.21 (s, 3H: NCH_3); from 3.30 to 3.50 (mt, 1H: the other H of the CH_2 in 4 β); from 3.30 to 3.50 and 3.63 (2 mts, 1H each: CH_2 in 3 δ); 4.53 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the

other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.88 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.35 (broad d, J = 6 Hz, 1H: 5 α); 5.43 (dd, J = 10. 5 and 4 Hz, 1H: 4 α); 5.90 (d, J = 9.5 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.56 (d, J = 9.5 Hz, 1H: NH in 2); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.28 (d, J = 8 Hz, 2H: aromatic H in 4 δ); 7.38 (dd, J = 8.5 and 2 Hz, 1H: $1'H_4$); 7.42 (dd, J = 8.5 and 4.5 Hz, 1H: $1'H_5$); 7.66 (dd, J = 4.5 and 2 Hz, 1H : $1'H_6$); 7.88 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

EXAMPLE 28: Preparation of 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-3-dimethylaminophenylalanine dihydrochloride, synthesized as in Example 35-10, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dri d ov r sodium sulphate and evaporated. Th dry extract is taken up in

20 ml of dichloromethan and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 57% 100 mM phosphate buffer, pH 2.9, and 43% of acetonitrile. The fractions containing 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 1.1 mg of 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.63 (dd, J = 16 and 5 Hz, 1H: 1H of the CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of the CH_2 in 3 β); from 1.20 to 1.35 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.76 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.08 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ): 2.35 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.81 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.90

(s, 6H: $N(CH_3)_2$); 2.97 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.20 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.28 (s, 3H: NCH_3); 3.37 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: th other H of the CH_2 in 3 δ); 4.58 (t, J = 7.5 Hz, 1H : 3 α); 4.74 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.86 (mt, 1H: 2 α); 4.89 (broad d, J = 10 Hz, 1H: 1 α); 5.27 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.29 (broad d, J = 5 Hz, 1H : 5 α); 5.89 (d, J = 9.5 Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.50 (d, J = 10 Hz, 1H: NH in 2); from 6.50 to 6.70 (mt, 3H: aromatic Hs in the ortho and in the para positions with respect to the dimethylamino); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.20 (t, J = [lacuna]

ml of a 20 g/l solution of (R,S)-3-methylthiophenylalanine hydrochloride, synthesized as in Example 34-11, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, th 1.68 litres of must recovered from the 56 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the novel derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 54% of water and 46% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% of acetonitrile. The fractions containing the novel pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 20 mg of 4ϵ-m thylthio-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.56 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of the CH_2 in 3 β); 1.28 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.58 (mt, 1H: the other H of the CH_2 in 3 γ); 1.62 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.25 and 2.35 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.39 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.43 (s, 3H: SCH_3); 2.82 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.98 (dd, J = 12 and 4.5 Hz, 1H: 1H of the CH_2 in 4 β); 3.26 (s, 3H: NCH_3); 3.30 (t, J = 12 Hz 1H: 1H of CH_2 in 3 δ); 3.38 (mt, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 4.56 (t, J = 7.5 Hz, 1H: 3 α); 4.74 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.29 (dd, J = 12 and 4.5 Hz, 1H : 4 α); 5.32 (broad d, J = 5.5 Hz, 1H : 5 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.51 (d, J = 10 Hz, 1H: NH in 2); 6.99 (broad d, J = 8 Hz, 1H: aromatic H in the para position with respect to the methylthio); 7.10 and 7.15 (respectively, broad s and broad d, J = 8 Hz, 1H each: aromatic Hs in the ortho position with respect to the methylthio); from 7.15 to 7.35 (mt, 6H: aromatic Hs in 6 and aromatic Hs in th meta position with respect to the methylthio); 7.43 (broad d, J = 8 Hz, 1H: 1'H_4);

7.52 (dd, J = 8 and 4 Hz, 1H: 1'H_5); 7.79 (broad d, J = 4 Hz, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.73 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 30: Preparation of 4ε-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-3-O-ethyltyrosine hydrochloride, synthesized as in Example 37-1, in 0.2N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the novel derivative of pristinamycin I_A are combined and evaporated. 19 mg of dry residue are obtained. The latter is taken up in 3 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a

semi-preparative Nucleosil 7μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitril . Th fractions containing th novel pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 15.8 mg of 4ϵ-O-ethoxy-de(4ζ-dimethyl-amino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.55 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.12 (mt, 1H: 1H of the CH_2 in 3 β); 1.20 (mt, 1H: 1H of the CH_2 in 3 γ); 1.31 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.49 (t, J = 7 Hz, 3H: CH_3 of the ethyl); 1.54 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.73 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.22 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.46 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.22 (mt, 1H: 1H of the CH_2 in 3 δ); 3.27 (s, 3H: NCH_3); 3.39 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.53 (mt, 1H: the other H of the CH_2 in 3 δ); 3.93 and 4.03 (2 mts, 1H each: OCH_2 of the ethyl); 4.56 (dd, J = 7 and 5.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.82 (mt, 1H: 2 α); 4.88 (dd, J = 10

and 1 Hz, 1H: 1 α); 5.23 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.23 (broad d, J = 5.5 Hz, 1H: 5 α); 5.87 (d, J = 9.5 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.47 (d, J = 10 Hz, 1H: NH in 2); 6.80 (mt, 3H: aromatic H in the ortho and in the para positions with respect to the ethoxy); from 7.10 to 7.35 (mt, 6H: aromatic Hs in 6 and aromatic Hs in the meta position with respect to the ethoxy); 7.43 (dd, J = 8 and 1 Hz, 1H: 1'H_4); 7.50 (dd, J = 8 and 4 Hz, 1H: 1'H_5); 7.77 (dd, J = 4 and 1 Hz, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.70 (d, J = 9.5 Hz, 1H: NH in 6); 11.60 (s, 1H: OH).

EXAMPLE 31: Preparation of 4ζ-ethylthio-de(4ζdimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-ethylthiophenylalanine hydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphat and evaporated. The dry extract is taken up in

20 ml of dichloromethane and inj cted onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethylthio-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-ethylthio-de(4ζ-dimethylamino)-pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. ? mg of 4ζ-ethylthio-de(4ζ-dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm): 0.68 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 5H: 1H of the CH_2 in 3 β and 1H of the CH_2 in 3 γ and CH_3 of the ethyl); 1.32 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.18 and 2.37 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.45 (broad d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.85 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.90 (mt, 2H:

$ArSCH_2$ ethyl); 2.98 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.25 (s, 3H: NCH_3); 3.35 (mt, 1H: 1H of the CH_2 in 3 δ); 3.39 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 4.55 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 7.5 Hz, 1H, : the other H of the CH_2 in 5 ϵ); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.25 to 5.40 (mt, 2H: 5 α and 4 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.55 (d, J = 9.5 Hz, 1H: NH in 2); 7.10 (d, J = 8 Hz, 2H: aromatic Hs in 4 δ); from 7.10 to 7.35 (mt, 7H: aromatic Hs in 6 and 4 ϵ); 7.44 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.74 (mt, 1H: $1'H_6$); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 32: Preparation of 4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylphenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times [lacuna] volumes of dichlorom thane. The

chloromethylene phases are wash d with water and then combined, dried over sodium sulphate and evaporated. Th dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 52% of water and 48% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 0.50 mg of 4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.42 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 2H: 1H of the CH_2 in 3 β and 1H of the CH_2 in 3 γ); 1.23 (t, J = 7.5 Hz, 3H: CH_3 of the ethyl); 1.35 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.15 and from 2.25 to 2.40 (2 mts, 1H each: CH_2 in 5 δ); from

2.25 to 2.40 (mt, 1H: the other H of the CH_2 in 5 β); 2.60 (q, J = 7.5 Hz, 2H: $ArCH_2$ of the ethyl); 2.83 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.98 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.25 to 3.35 (mt, 1H: 1H of the CH_2 in 3 δ); 3.27 (s, 3H: NCH_3); 3.39 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.59 (mt, 1H: the other H of the CH_2 in 3 δ); 4.58 (dd, J = 7 and 6.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.87 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.24 (broad d, J = 5.5 Hz, 1H: 5 α); 5.29 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.88 (d, J = 10 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.73 (d, J = 10 Hz, 1H: NH in 2); from 7.10 to 7.35 (mt, 9H: aromatic Hs in 6 - 4 ϵ and 4 δ); 7.44 (dd, J = 8.5 and 1.5 Hz, 1H: 1'H_4); 7.50 (dd, J = 8.5 and 4.5 Hz, 1H: 1'H_5); 7.80 (dd, J = 4.5 and 1.5 Hz, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH in 6); 11.66 (s, 1H: OH).

Using the same fractions derived from the silica column described above, which fractions also contain the novel pristinamycin I_H derivative, 0.3 mg of ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_H is isolated by carrying out semi-preparative column chromatography as described above.

NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm): 0.04 (mt 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 2H: 1H of the

CH_2 in 5 δ and 1H of the CH_2 in 5 γ); 1.18 (t, J = 7.5 Hz, 3H: CH_3 of the ethyl); 1.30 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 7H: the other H of the CH_2 in 5 γ - the other H of the CH_2 in 5 δ - 1H of the CH_2 in 3 β - CH_2 in 3 γ and CH_2 in 2 β); 1.81 (broad d, J = 13 Hz, 1H: the other H of the CH_2 in 5 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.40 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.65 (q, J = 7.5 Hz, 2H: $ArCH_2$ of the ethyl); 2.97 and 3.09 (respectively, dd and t, J = 12 and 5 Hz and J = 12 Hz, 1H each: CH_2 in 4 β); 3.50 and 3.60 (2 mts, 1H each: CH_2 in 3 δ); 4.13 (dd, J = 8 and 5 Hz, 1H: 3 α); 4.49 (broad d, J = 13 Hz, 1H: the other H of the CH_2 in 5 ε); 4.70 (mt, 2H: 5 α and 4 α); 4.77 (mt, 1H: 2 α); 4.83 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.50 (d, J = 7 Hz, 1H: 6 α); 5.74 (mt, 1H: 1 β); 6.09 (d, J = 4 Hz, 1H: NH in 4); 6.72 (unres. comp., 1H: NH in 2); 7.07 (d, J= 8 Hz, 2H: aromatic Hs in 4 ε); 7.15 (d, J = 8 Hz, 2H: aromatic Hs in 4 δ); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.40 (dd, J = 8 and 1 Hz, 1H: $1'H_4$); 7.45 (dd, J = 8 and 4 Hz, 1H: $1'H_5$); 7.92 (dd, J = 4 and 1 Hz, 1H: $1'H_6$); 8.40 (unres. comp., 1H: NH in 6); 8.50 (d, J = 10 Hz, 1H: NH in 1); 11.72 (s, 1H: OH).

EXAMPLE 33: Preparation of derivatives of phenylalanine and of phenylpyruvic acid which have already been described.

Phenylalanine, and its derivatives

4-methoxyphenylalanine, 4-bromophenylalanine, 4-chlorophenylalanine, 4-iodophenylalanine, 4-trifluoromethylphenylalanine, 4-aminophenylalanine and 3-methoxyphenylalanine, which are employed in this work, are commercially available.

The following derivatives of phenylalanine can be prepared in accordance with methods described in the literature.

(RS)-4-dimethylaminophenylalanine

D.F. Elliott, A.T. Fuller, C.R. Harrington, J. Chem. Soc., 1948, 85-89.

(RS)-4-diethylaminophenylalanine

Moldaver B.L., Pushkareva Z.V., Zhur. Obshchei Khim., 31, 1560-1569 (1961); C.A. 1961, 22226f.; J.A. Stock, J. Chem. Soc, 1959, 90-97

(RS)-4-ethylaminophenylalanine

F. Bergel, J.A. Stock, J. Chem. Soc, 1959, 90-97.

(RS)-4-phenylphenylalanine

J.V. Braun, J. Nelles, Berichte, 66B, 1933, 1464-1470.

(RS)-4-methylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dailey, J. Am. Chem. Soc, 1957, 79, 4229-4231.

(RS)-4-methylthiophenylalanine and (R,S)-4-ethylthiophenylalanine

R.L. Colescott, R.R. Herr, J.P. Dailey J. Am. Chem. Soc, 1957, 79, 4232-4235.

(RS)-4-methoxycarbonylphenylalanine

H. Cleland, J. Org. Chem., 1969, 34, 747.

(RS)-2,4-dimethylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dail y, J. Am. Chem. Soc, 1957, 79, 4229-4231.

(RS)-3,4-dimethylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dailey, J. Am. Chem. Soc, 1957, 79, 4229-4231.

(RS)-3-trifluoromethylphenylalanine hydrochloride

R. Filler and H. Novar. J. Org. Chem, 1960, 25, 733-736.

(S)-4-aminomethylphenylalanine

G.E. Stokker, W.F. Hoffman and C.F. Homnick, J. Org. Chem., 1993, 58, 5015-5017.

(R,S)-3-methylphenylalanine

J.H. Burckhalter, V.C. Stephens, J.A.C.S. 1951, 73, 56-58.

(R,S)-4-acetylphenylalanine

J.I. Degaw et coll., J. Med. Che., 1969, 11, 225-227

(S)-4-O-allyltyrosine

A. Loffet, H. Zang, Int. J. Pept. Protein. Res., 1993, 42, 346

(S)-4-O-ethyltyrosine

Y. Sasaki et coll., Chem. PLarm, Bull., 1982, 30, 4435

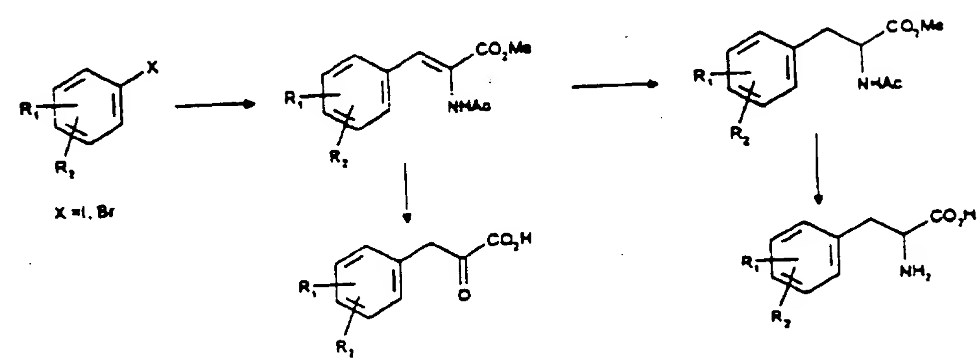
(RS)-4-ethylphenylalanine

A. Zhuze et coll., Coll., Czech. Chem. Commmm., 1965, 62, 2648

4-tert-butylphenylpyruvic acid can be prepared in accordance with R. Breslow, J.W. Canary, M. Varney, S.T. Waddell and D. Yang, J. Am. Chem. Soc., 1990, 112, 5212-5219.

The other derivatives of phenylalanine were prepared in accordance with Examples 34 to 42 which are given below. In these examples, flash chromatography was carried out under a mean nitrogen pressure of 50 kPa using a silica of granule size 40-53 μm, in accordance with Still et al., J. Org. Chem., 43, 2923, (1978).

EXAMPLE 34: Preparation of derivatives of phenylalanine and of a derivative of phenylpyruvic acid using method A.



34-1 <u>(RS)-4-methylaminophenylalanine, dihydrochloride</u>

37 ml of 12 N hydrochloric acid are added to 3.70 g of methyl N-acetyl-4-methylaminophenylalaninate, and the mixture is heated to reflux, while stirring,

for 8 h. Aft r on night at room temp rature, the reaction medium is concentrated to dryness under reduced pressure (50 kPa), and the residu is taken up in a mixture of 50 ml of toluen and 50 ml of ethanol, and this mixture is concentrated once again. After drying in a desiccator under reduced pressure (2.6 kPa), 4.18 g (100%) of (RS)-4-methylaminophenylalanine dihydrochloride are obtained in the form of a hygroscopic light beige solid which melts at 158°C.

34-2: <u>Methyl (RS)-N-acetyl-4-methylaminophenylalaninate</u>

0.4 g of 10% palladium on charcoal, and then 50 ml of absolute ethanol, are added to 4 g of methyl 4-methylamino-2-acetamidocinnamate which is placed under a nitrogen atmosphere in an autoclave. The mixture is placed under a pressure of 5.5 bar of hydrogen and heated at 50°C for 15 h with stirring. After stabilizing the temperature at 26°C, and returning the pressure to atmospheric, the medium is filtered through Clarcel®, washed with ethanol and then concentrated to dryness under reduced pressure (2.6 kPa). This results in 3.73 g of methyl N-acetyl-4-methylaminophenylalaninate in the form of white crystals which melt at 118°C.

34-3: <u>Methyl 4-methylamino-2-acetamidocinnamate</u>

5.75 g of methyl 2-acetamidoacrylate, 0.185 g

of palladium ac tate, 8.1 g of tetrabutylammonium chloride and 6.03 g of sodium hydrogen carbonate are added to a 3-necked flask which is placed under nitrogen, and then 6.5 g of 4-iodo-N-methylalanine, in solution in 200 ml of DMF, are added to this mixture. The mixture is heated at 82°C for 16 h 30 min and then, after having been cooled down, is poured into 1000 ml of distilled water. The medium is extracted with 250 ml of CH_2Cl_2 and the organic phase is separated off; the aqueous phase is then washed twice with 250 ml of CH_2Cl_2. The organic phases are combined, dried over sodium sulphate, filtered and concentrated under reduced pressure (50 kPa) at 70°C to yield a brown oil which is purified by flash chromatography (eluent, AcOEt/cyclohexane and then pure AcOEt).

In this way, 4 g of methyl 4-methylamino-2-acetamidocinnamate is obtained in the form of a yellow solid (Merck Silica 5719, R_f = 0.48), which is employed in this form.

N-Methyl-p-iodoanaline can be prepared in accordance with: S. Krishnamurthy, Tetrahedron Letters, 33, 3315-3318, 1982.

34-4: <u>4-methylaminophenylpyruvic acid</u>

2.4 g of methyl 4-methylamino-2-acetamidocinnamate and 32 ml of 12 N hydrochloric acid are placed in a round-bottomed flask. The mixture is heated to reflux for 3 h and then cooled down and washed twice with 20 ml of diethyl ether. The aqueous

phas is cooled down to -10°C and the pr cipitate which is obtained is filtered and then rinsed with a minimum of cold hydrochloric acid. The solid which is obtained is dried in a desiccator under reduced pressure in order to yield 1.1 g of 4-methylaminophenylpyruvic acid in the form of a light beige solid which melts at 210°C.

34-5: <u>(R,S)-3-Fluoro-4-methylphenylalanine hydrochloride</u>

0.6 g of (R,S)-3-fluoro-4-methylphenylalanine hydrochloride is obtained in the form of white crystals which melt at a temperature greater than 260°C by proceeding as in Example 34-1 but using 1.6 g of methyl N-acetyl(3-fluoro-4-methyl)phenylalaninate.

34-6: <u>Methyl (R,S)-N-acetyl-(3-fluoro-4-methyl)phenylalaninate</u>

1.6 g of methyl N-acetyl-(3-fluoro-4-methyl)phenylalaninate are obtained in the form of a colourless oil (Merck Silica 5719, R_f =0.46; eluent CH_2Cl_2/AcOEt 50/50), by proceeding as in Example 34-2 but using 1.9 g of methyl (4-methyl-3-fluoro)-2-acetamidocinnamate and 0.2 g of 10% palladium on charcoal in 230 ml of ethanol.

34-7: <u>Methyl (3-fluoro-4-methyl)-2-acetamidocinnamate</u>

2.6 g of methyl (3-fluoro-4-methyl)-2-acetamidocinnamate are obtained in th form of a white solid which melts at 163°C by proceeding as in Example

34-3 but using 3.6 g of m thyl 2-acetamidoacrylate, 0.12 g of palladium acetate, 5.2 g of tetrabutylammonium chloride, 3.8 g of sodium hydrogen carbonate and 4 g of 2-fluoro-4-bromotoluene in solution in 120 ml of anhydrous DMF.

34-8: <u>(R,S)-4-Trifluoromethoxyphenylalanine hydrochloride</u> or <u>(R,S)-O-trifluoromethyltyrosine hydrochloride</u>

1.5 g of (R,S)-4-trifluoromethoxyphenylalanine hydrochloride are obtained in the form of white crystals which melt at 260°C by proceeding as in Example 34-1 but using 3 g of methyl N-acetyl-(4-trifluoromethoxy)phenylalaninate and 30 ml of 12 N hydrochloric acid.

34-9: <u>Methyl (R,S)-N-acetyl-(4-trifluoromethoxy)phenylalaninate</u>

3 g of methyl N-acetyl-(4-trifluoroethoxy)-phenylalaninate are obtained in the form of a white solid which melts at 80°C by proceeding as in Example 34-2 but using 3.1 g of methyl (4-trifluoromethoxy)-2-acetamidocinnamate and 0.3 g of 10% palladium on charcoal in 50 ml of ethanol.

34-10: <u>Methyl 4-trifluoromethoxy-2-acetamidocinnamate</u>

3.1 g of methyl (4-trifluoromethoxy)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 135°C by proceeding as in Example 34-3 but using 4.3 g of methyl 2-acetamido acrylate,

0.14 g of palladium ac tat , 6.1 g of tetrabutyl-mmonium chloride, 4.6 g of sodium hydrogen carbonate and 5 g of 4-trifluoromethoxybromobenzen in solution in 150 ml of anhydrous DMF.

34-11: <u>(R,S)-3-Methylthiophenylalanine hydrochloride</u>

1.38 g of (R,S)-3-methylthiophenylalanine hydrochloride are obtained in the form of white crystals which melt at 190°C by proceeding as in Example 34-1 but using 3.3 g of methyl N-acetyl-3-methylthiophenylalaninate and 40 ml of 12 N hydrochloric acid.

34-12: <u>Methyl (RS)-N-acetyl-3-methylthiophenylalaninate</u>

3.72 g of methyl 3-methylthio-2-acetamidocinnamate, dissolved in 100 ml of methanol, and 30 ml of tetrahydrofuran are placed in a round-bottomed flask, and 1.4 g of magnesium are then added. After reacting for 20 min, the mixture is cooled in an ice bath and a further 1.4 g of magnesium are then added. The mixture is stirred at room temperature for 18 h and then poured into 1.4 l of distilled water and 300 ml of CH_2Cl_2; this mixture is then filtered through Clarcel®. The aqueous phase is adjusted to pH 6 by adding 12 N hydrochloric acid and then separated off and washed with 100 ml of CH_2Cl_2. The organic phases are collected, dried over magnesium sulphate, filtered and then concentrated to dryn ss under reduced pressure in

order to yi ld 3.42 g of methyl N-ac tyl-3-m thylthiophenylalaninat in the form of a colourless oil (Merck Silica 5719, R_f=0.5; AcOEt).

34-13: <u>Methyl 3-methylthio-2-acetamidocinnamate</u>

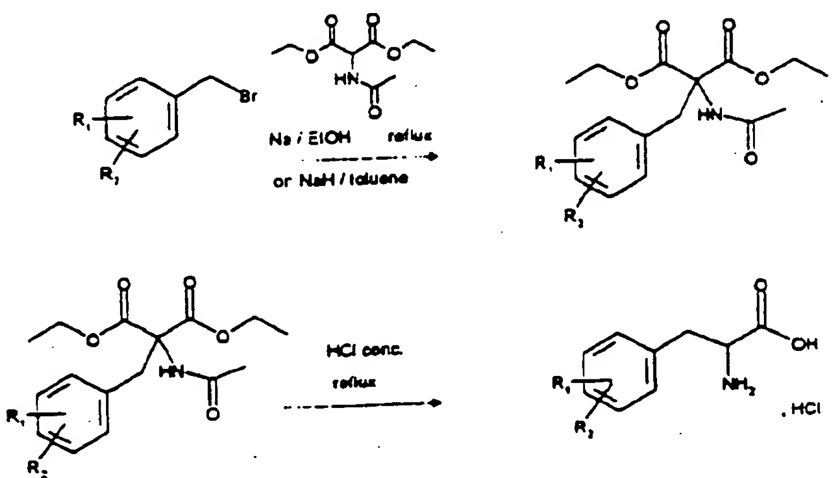
4.8 g of methyl (3-methylthio)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 139°C by proceeding as in Example 34-3 but using 5.6 g of methyl 2-acetamidoacrylate, 0.18 g of palladium acetate, 8.2 g of tetrabutylammonium chloride, 5.86 g of sodium hydrogen carbonate and 6.5 g of 3-iodo-1-methylthiobenzene dissolved in 160 ml of anhydrous DMF.

34-14: <u>3-Iodomethylthiobenzene</u>

20 ml of distilled water and 20 ml of 12 N hydrochloric acid are placed, with stirring, in a three-necked flask, and 10 ml of 3-methylthioaniline are then added using a dropping funnel. The mixture is warmed to ensure dissolution and is then cooled down to 5°C. 5.86 g of sodium nitrite dissolved in 15 ml of water are subsequently added slowly, using a dropping funnel, while maintaining the temperature between 5 and 8°C. 20 min after having completed the addition, 13.57 g of potassium iodide dissolved in 15 ml of water are added over a period of 10 min and the mixture is then stirred at room temperature for 15 h. The oil which forms is separated from the aqueous phase by decantation, and an aqueous solution of sodium

thiosulphate is th n added to it. The aqueous phase is decant d and the product is extracted with 100 ml of dichloromethane. The organic phas is washed with 100 ml of water, and the aqueous phase is adjusted to pH 9 with concentrated sodium hydroxide solution, and then separated off. The organic phase is washed with 2 times 100 ml of water, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure (50 kPa) at 40°C. The resulting product is purified by flash chromatography (eluent, cyclohexane) in order to yield 13 g of 3-iodo-1-methylthiobenzene in the form of a yellow liquid (Merck Silica 5719, R_f=0.8/cyclohexane).

EXAMPLE 35: Preparation of derivatives of phenylalanine using method B.



35-1: <u>(RS)-4-tert-butylphenylalanine</u>

25 g of diethyl 4-(tert-butyl)benzyl acetamidomalonate and 250 ml of 37% hydrochloric acid are added to a three-necked flask which is surmounted

by a cond nser. The mixture is stirr d and heated to reflux until there is no furth r evolution of gas. After the reaction medium has been cooled down, the precipitate which is obtained is filtered and then recrystallized in acetonitrile to yield 25.6 g of (R,S)-4-tert-butylphenylalanine hydrochloride in the form of a white solid which melts at 234°C.

35-2: Diethyl 4-(tert-butyl)benzylacetamidomalonate

25 g of 4-(tert-butyl)benzyl bromide, 50 ml of anhydrous toluene and 3.1 g of sodium hydride in 80% suspension in oil are added to a three-necked flask which is surmounted by a condenser, followed by 21.8 g of diethyl acetamidomalonate. The mixture is heated at 110°C for 17 h. After it has been cooled down, 15 ml of absolute ethanol, then 15 ml of 50% ethanol and then 50 ml of water are added slowly to it using a dropping funnel. The organic phase is decanted and the aqueous phase is washed with 3 times 50 ml of diethyl ether. The organic phases are combined, washed with water and then dried over sodium sulphate. Following filtration and concentration under reduced pressure, the product is crystallized in petroleum ether in order to yield 25 g of diethyl 4-(tert-butyl)benzylacetamidomalonate in the form of a white solid which melts at 80°C.

35-3: (R,S)-3-Methylaminophenylalanine dihydrochloride

1.03 g of a yellow-beige solid are obtained

by proceeding as in Example 35-1 but using 1.17 g of diethyl 3-methylaminobenzylacetamidomalonate and 20 ml of 12 N hydrochloric acid. This yellow-beige solid is dissolved in 20 ml of absolute ethanol, and 0.4 g of animal charcoal is added to this solution. The solution is filtered through Clarcel and then filtered and concentrated under reduced pressure (50 kPa). The same procedure is repeated starting with 1 g of animal charcoal, and the solid which is obtained is triturated in 20 ml of ether. Following filtration and drying under reduced pressure (2.7 kPa) at 50°C, 0.65 g of (R,S)-3-methylaminophenylalanine dihydrochloride is obtained in the form of a white powder which melts at a temperature approaching 135°C (decomposition).

35-4: <u>Diethyl 3-methylaminobenzylacetamido-malonate</u>

3.11 ml of acetic anhydride are placed in a three-necked flask which is maintained under a nitrogen atmosphere. 1.51 ml of formic acid are subsequently added within 3 min at 0°C, and the mixture is then heated at 50°C for 2 hours. The mixture is allowed to return to room temperature, while shaking for 3 h 20 min, and 4 ml of anhydrous THF are added under nitrogen; the mixture is then cooled to -20°C. A solution of 4 g of diethyl 3-aminobenzylacetamidomalonate in a mixture of 15 ml of anhydrous THF and 15 ml of anhydrous dichloromethane is added within 10 min. Stirring is continued for 1 h

10 min at -20°C and then for 16 h at 20°C. The reaction mixture is concentrated to dryness under reduced pr ssure (50 kPa) at 30°C and then co-evaporated with 30 ml of anhydrous toluene in order to yield a white solid, which is dissolved in a mixture of 10 ml of anhydrous THF and 20 ml of anhydrous 1,2-dichloroethane, which solution is then placed in a three-necked flask under nitrogen.

The medium is cooled down to -5°C, and 1.55 ml of borane-dimethyl sulphide complex (2M solution in THF) are then added within 10 min. The mixture is allowed to return to room temperature, and the solution is heated to reflux for 3 h and then stirred at room temperature for 15 h. The reaction medium is cooled to 0°C, and 10 ml of MeOH are then added within 25 min. The mixture is stirred for 45 min at 0°C and then for 30 min at room temperature. It is then cooled to 0°C and HCl gas is bubbled in until a pH of 2 is reached. The mixture is heated at reflux for 1 h and is then concentrated to dryness under reduced pressure at 30°C in order to yield 5 g of a product which is taken up in 30 ml of an aqueous solution of $NaHCO_3$ and 30 ml of CH_2Cl_2. The organic phase is decanted and the aqueous phase is washed with 20 ml of water. The organic phases are pooled, dried over magnesium sulphate, filtered and then concentrated tc dryness under reduced pr ssure (2.6 kPa) in order to yield 3.43 g of a yellow oil, which is purified by

flash chromatography (eluent, AcOEt/cyclohexane 50/50). After drying under reduc d pressure (2.7 kPa) at 20°C, 1.18 g of diethyl 3-methylaminobenzylacetamidomalonate are thus obtained in the form of a light beige solid which melts at 122°C.

35-5: Diethyl 3-aminobenzylacetamidomalonate

Diethyl 3-aminobenzylacetamidomalonate can be prepared as described in:

T.S. Osdene, D.N. Ward, W.H. Chapman and H. Rakoff, J. Am. Chem. Soc., 81, 1959, 3100-3102.

35-6: (R,S)-3-Ethylaminophenylalanine dihydrochloride

1.7 g of (R,S)-3-ethylaminophenylalanine dihydrochloride are obtained in the form of a hygroscopic light beige solid, which contains 10 molar % of (R,S)-3-diethylaminophenylalanine dihydrochloride, by proceeding as in Example 34-1 but using 2 g of ethyl (R,S)-N-acetyl-3-ethylaminophenylalaninate and 30 ml of 12N hydrochloric acid.

35-7: (R,S)-N-acetyl-3-ethylaminophenylalaninate

3 g of ethyl (R,S)-N-acetyl-3-aminophenylalaninate, 40 ml of ethanol and 14 g of Raney nickel, which has previously been washed with distilled water and ethanol, are placed in a round-bottomed flask under a nitrogen atmosphere. The mixture is heated to reflux for 19 h, cooled down, filtered through Clarcel®, and then concentrated to dryn ss under r duc d pressur

(50 kPa) in order to yield 3.07 g of a colourless oil, which is purified by flash chromatography (eluent, AcO t) in ord r to yield 2.1 g of thyl (R,S)-N-acetyl-3-ethylaminophenylalaninate in the form of a colourless oil (Merck Silica 5719, R_f=0.6: AcOEt) which contains 10% ethyl (R,S)-N-acetyl-3-diethylaminophenylalaninate.

35-8: <u>Ethyl (R,S)-N-acetyl-3-aminophenylalaninate</u>

25 g of a mixture of ethyl (R,S)-N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl 3-nitrobenzylacetamidomalonate (25 mol %/mol) are placed under nitrogen in an autoclave. 2.5 g of 10% palladium on charcoal and then 200 ml of dichloromethane are added. The mixture is placed under a hydrogen pressure of 9 bar and then stirred at 18°C for 4 h. After returning the pressure to atmospheric, the reaction medium is filtered through Clarcel®, washed with dichloromethane and then concentrated to dryness under reduced pressure (50 kPa) in order to yield a solid, which is recrystallized in 450 ml of distilled water under reflux and in the presence of 4 g of 3S animal charcoal. Following hot filtration through Clarcel®, the mixture is left to crystallize at 4°C, with the crystals being filtered and then dried in order to yield 9.9 g of ethyl (R,S)-N-acetyl-3-aminoph nylalaninate in the form of a light beige solid which melts at 106°C and which contains 5% of diethyl 3-aminobenzylacetamidomalonate.

35-9: Ethyl (R,S)-N-acetyl-3-nitrophenylalaninate and diethyl 3-nitrobenzylacetamidomalonate

600 ml of absolute ethanol and then 7.9 g of sodium are placed, under a nitrogen atmosphere, in a three-necked flask which is surmounted by a condenser. Once dissolution is complete, 74.5 g of diethyl acetamidomalonate and then 60 g of 4-nitrobenzyl chloride in 200 ml of anhydrous ethanol are added. The mixture is heated to reflux for 16 h 30 min. After cooling, the reaction medium is concentrated under reduced pressure (50 kPa) and then taken up in a mixture of 500 ml of CH_2Cl_2 and 500 ml of water. The pH is adjusted to 7 by adding 0.5N sulphuric acid, and the organic phase is then separated off and the aqueous phase is washed with 2 times 200 ml of CH_2Cl_2. The organic phases are pooled, washed with 200 ml of water saturated with sodium bicarbonate, separated off and then dried over magnesium sulphate. Following filtration and concentration under reduced pressure (50 kPa), the product is recrystallized in 600 ml of ethanol at reflux in order to yield, after crystallizing at ambient temperature, filtering and drying, 70.4 g of diethyl 3-nitrobenzylacetamidomalonate in the form of white crystals which melt at 156°C. The mother liquors are concentrated and then purified by flash chromatography (eluent, AcOEt) in order to yield 25.6 g of a mixture of ethyl N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl

3-nitrobenzylacetamidomalonate (25 mol %/mol) in the form of a light beige solid, which is used in this form in the following step.

35-10: <u>(RS,)-3-Dimethylaminophenylalanine dihydrochloride</u>

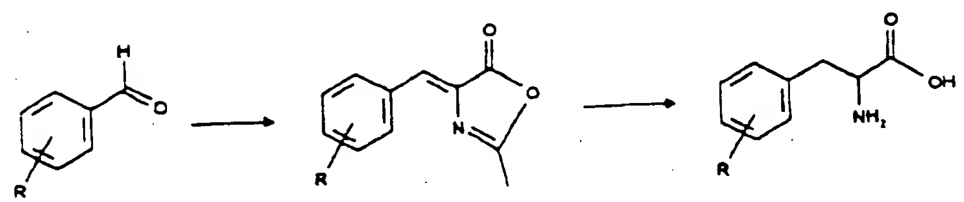
A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 0.72 g of ethyl (RS)-N-acetyl-3-dimethylaminophenylalaninate and 8.6 ml of 10N hydrochloric acid; the solid is subsequently triturated in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 0.68 g (93%) of (RS)-3-dimethylaminophenylalanine dihydrochloride is obtained in the form of a white solid which melts in the region of 120°C (decomposition).

35-11: <u>Ethyl (RS)-N-acetyl-3-dimethylaminophenylalaninate</u>

4 g of ethyl (RS)-N-acetyl-3-aminophenylalaninate, prepared as described in Example 35-8, in 15 ml of DMF are placed in a three-necked flask under a nitrogen atmosphere, and 5.5 ml of triethylamine, and then 2.5 ml of methyl iodide and 4 ml of dichloromethane, are added while maintaining the temperature in the region of 30°C using an icebath. The mixture is then warmed at 35°C for 18h. 1 ml of methyl iodide dissolved in 1 ml of DMF is then added slowly while maintaining the temperature in the region of 30°C; 2.2 ml of triethylamine are then added and the mixture is subsequently warmed for a further 5h at

35°C. The mixture is brought to room temperature and then extracted with 100 ml of ethyl ac tate and 150 ml of distilled water. The aqu ous phase is separated off after settling and then rewashed with 2 times 70 ml of ethyl acetate. The organic phases are combined, washed with 2 times 80 ml of distilled water and then with 50 ml of distilled water which is saturated with NaCl. The organic phase is separated off after settling, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 2.4 g of a product which is purified by flash chromatography (dichloromethane, MeOH 90/10). 0.72 g (16%) of ethyl (RS)-3-N-acetyl-3-dimethylamino phenylalaninate is thus obtained in the form of yellow crystals.

EXAMPLE 36: Preparation of derivatives of phenylalanine using method C.



36-1: <u>(R,S)-4-Isopropylphenylalanine</u>

7 g of red phosphorus and 8 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone, in 47 ml of acetic anhydride, are placed in a three-necked flask, and then 35 ml of 57% hydriodic acid are added slowly, with stirring, using a dropping funnel. Once

the addition is complete, the mixture is heated to reflux for 3 h 30 min and then left at room temperature for 3 days. The reaction mixture is filtered and the solid which is obtained is rinsed twice with 10 ml of acetic acid on each occasion, and the filtrate is then concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water, and this solution is concentrated to dryness under reduced pressure in order to yield a solid which is taken up in 50 ml of distilled water; this solution is then extracted with 3 times 50 ml of diethyl ether after 0.5 g of sodium sulphite have been added. The ether is separated off and the aqueous phase is placed under reduced pressure in order to eliminate traces of diethyl ether. 2 g of animal charcoal are added to the aqueous phase, which is heated at 40-50°C, and then filtered through Clarcel®; rinsing then takes place with a minimum of water. The pH is adjusted to 5 by adding 32% ammonia at 4°C. The precipitate which is obtained is filtered in the cold, rinsed with 2 times 10 ml of water, with 10 ml of ethanol and then with 2 times 10 ml of ether in order to yield, after drying under reduced pressure at 20°C, 3.97 g of (R,S)-4-isopropylphenylalanine in the form of a white solid which melts at a temperature greater than 260°C. (See also Journal of the Takeda Research Laboratories, vol. 43; nos. 3/4, Dec. 1984, pp 53-76).

36-2: 4-(Isopropylbenzylidene)-2-methyl-5-oxazolone

18.52 g of N-acetylglycine, 10.6 g of sodium acetate, 20 ml of 4-isopropylbenzaldehyde and 57 ml of acetic anhydride are placed in a round-bottomed flask which is provided with a condenser. The mixture is stirred for 30 min and then stirred for 1 h at 110°C and subsequently for 15 h at room temperature. The reaction medium is poured into 600 ml of water and 400 ml of petroleum ether which has previously been heated to 50°C. The organic phase is separated off and the aqueous phase is washed with 2 times 150 ml of petroleum ether.

The organic phases are combined, dried over magnesium sulphate, filtered and concentrated under reduced pressure until the volume is 100 ml and a precipitate is obtained. The latter is filtered and washed with 2 times 50 ml of pentane in order to yield 8.2 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone in the form of a yellow solid which melts at 77°C.

36-3: (R,S)-4-Butylphenylalanine

0.35 g of (R,S)-4-butylphenylalanine is obtained in the form of a light beige solid which melts at a temperature greater than 260° by proceeding as in Example 36-1 but using 1.49 g of red phosphorus, 1.8 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone, in 9.23 ml of acetic anhydride, and 7.39 ml of 57% hydriodic acid.

36-4: <u>4-(Butylbenzylidene)-2-methyl-5-oxazolone</u>

1.89 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone are obtained in the form of a yellow solid which melts at 74°C by proceeding as in Example 36-2 but using 8.43 g of N-acetylglycine, 4.92 g of sodium acetate, 9.8 g of 4-butylbenzaldehyde and 26 ml of acetic anhydride.

EXAMPLE 37: Preparation of a derivative of phenylalanine using method D.

37-1: <u>(R,S)-3-Ethoxyphenylalanine hydrochloride (or (R,S)-3-O-ethyltyrosine hydrochloride</u>)

1 g of (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalanine, dissolved in 3.6 ml of hydrochloric dioxane, is placed in a round-bottomed flask, and the mixture is then stirred at room temperature for 5 h. The precipitate which forms is filtered, rinsed with dioxane and then ether, and then dried under reduced pressure (2.7 kPa) at 40°C to yield 0.65 g of (R,S)-3-ethoxyphenylalanine hydrochloride in the form of a white solid which melts at 200°C.

37-2: <u>(R,S)-N-tert-Butoxycarbonyl-3-ethoxyphenylalanine</u>

1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalaninate, dissolved in 8 ml of methanol, are placed in a round-bottomed flask, and 8 ml of 1N sodium hydroxide solution are then added. After the

mixture has been stirred at room temperature for 18 h, it is evaporated under reduced pressure and then acidified with 8.56 ml of 1N hydrochloric acid. The product is extracted with 2 times 10 ml of ethyl acetate, and the organic phases are pooled, washed with 2 times 10 ml of water, dried, filtered and then concentrated to dryness under reduced pressure to yield 1 g of (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalanine in the form of a yellow oil (Merck Silica 5719, R_f=0.7, eluent: toluene 80/MeOH 10/diethylamine 10).

37-3: <u>(R,S)-N-tert-Butoxycarbonyl-3-ethoxyphenylalaninate</u>

1.5 g of (R,S)-N-tert-butoxycarbonyl-3-tyrosine, dissolved in 7.5 ml of dry DMF, are placed in a three-necked flask under a nitrogen atmosphere, and 0.508 g of sodium hydride, as a 50% dispersion in oil, is then added. After the mixture has been stirred at room temperature for 2 h, 0.86 ml of iodoethane is added and the mixture is then stirred at room temperature for 4 h. The medium is filtered and the resulting solid is washed with 3 times 10 ml of water and then 2 times 10 ml of petroleum ether to yield, after drying under reduced pressure (2.7 kPa) at 30°C, 1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalaninate in th form of a white solid.

37-4: <u>(R,S)-N-tert-Butoxycarbonyl-3-tyrosine</u>

18 g of (R,S)-3-tyrosine, dissolved in 180 ml

of dioxane, are placed, with stirring, in a three-necked flask, and 99 ml of 1N sodium hydroxide solution, followed by 26 g of di-tert-butyl dicarbonate, dissolved in 160 ml of dioxane, are then added. After the mixture has been stirred for 36 h, it is concentrated under reduced pressure at 30°C and the residue is taken up in 100 ml of distilled water; this solution is acidified to pH 5 with 1N hydrochloric acid and then extracted with 2 times 200 ml of ethyl acetate. The organic phase is dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure at 30°C to yield 30 g of (R,S)-N-tert-butoxycarbonyl-3-tyrosine in the form of a white solid (Merck Silica 5719, R_f=0.25, eluent: toluene 80, MeOH 10, diethylamine 10).

EXAMPLE 38: Preparation of derivatives of phenylalanine using method E.

38-1: <u>(RS)-4-Diallylaminophenylalanine dihydrochloride</u>

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 5.8 g of diethyl 4-diallylaminobenzylacetamido malonate and 48 ml of 10N hydrochloric acid; the solid is then triturated in 50 ml of acetone, filtered, then triturated in 10 ml of dichloromethane, filtered and then rinsed with 3 times 10 ml of ethyl ether. After drying under reduced pressure (2.7 kPa) at 40°C, 4.41 g

of (RS)-4-diallylaminophenylalanine dihydrochloride are obtained in the form of an off-white solid which melts in the region of 135°C (decomposition).

38-2: <u>(RS)-4-Allylaminophenylalanine dihydrochloride</u>

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 3.27 g of diethyl 4-allylaminobenzylacetamidomalonate and 30 ml of 10N hydrochloric acid; the solid is then triturated in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.3 g of (RS)-4-allylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 134°C (decomposition).

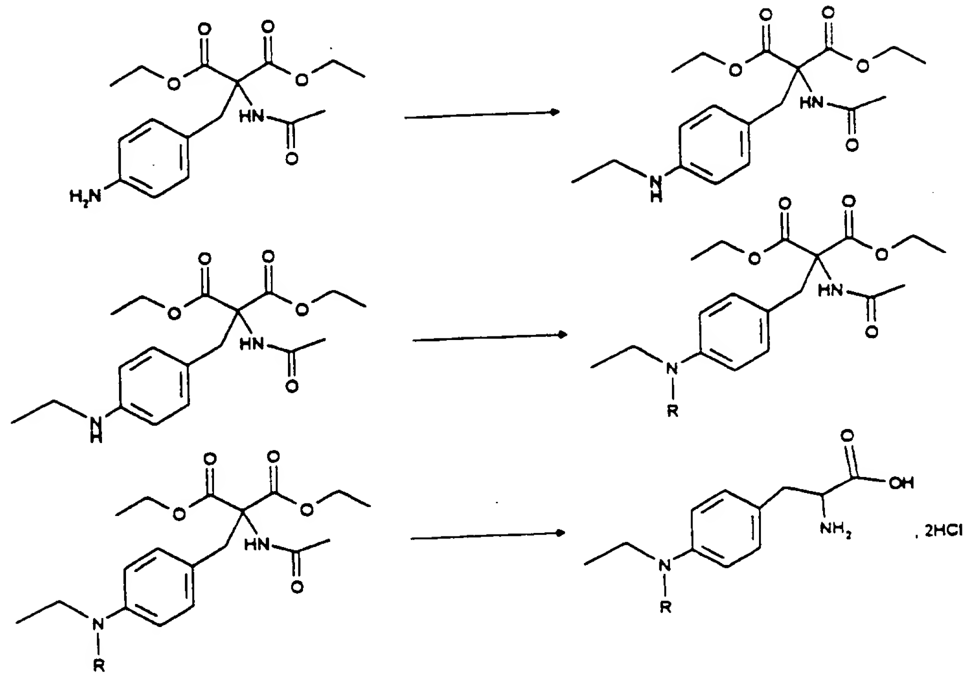
38-3: <u>Diethyl 4-diallylaminobenzylacetamidomalonate and diethyl 4-allylaminobenzylacetamidomalonate</u>

10 g of diethyl 4-aminobenzylacetamidomalonate dissolved in 150 ml of DMF are placed in a three-necked flask which is surmounted with a dropping funnel and maintained under a nitrogen atmosphere. 6.57 ml of allyl bromide, and then 10.76 ml of triethylamine, are added slowly, at room temperature and while stirring. After stirring for 19h, a further 1.31 ml of allylbromide and 2.15 ml of triethylamine are then added and the mixture is stirred for 26h. The reaction medium is poured onto 1.5 l of distilled water and this mixture is extracted with 1 l of ethyl

acetate. The aqueous phase is separated off after settling and washed with 2 times 500 ml of ethyl acetate. The organic phas s are combined, washed with 500 ml of distilled water and then with 500 ml of water which is saturated with sodium chloride, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness in order to yield a chestnut oil; this oil is purified by flash chromatography (eluant, $CH_2Cl_2$90/AcOEt 10) in order to yield 6.66 g of diethyl 4-diallylaminobenzylacetamidomalonate in the form of a beige solid which melts at 94-96°C (Rf = 0.6, AcOEt 50/cyclohexane 50) and 3.49 g of diethyl 4-allylaminobenzylacetamidomalonate in the form of a beige solid which melts at 104-106°C (Rf = 0.45 AcOEt 50/cyclohexane 50).

The diethyl 4-aminobenzylacetamidomalonate can be prepared as described in J.B. Burckhalter, VC Stephens, J. Am. Chem. Soc. 56, 1951, 73.

EXAMPLE 39: Preparation of derivatives of phenylalanine using m thod F



39-1: <u>(RS)-4-ethylisopropylphenylalanine dihydrochloride</u>

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.9 g of diethyl 4-ethylisopropylbenzylacetamidomalonate and 24.6 ml of 10N hydrochloric acid; the solid is then triturated in 20 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2 g of (RS)-4-ethylisopropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).

39-2: Diethyl 4-ethylisopropylaminobenzyl-acetamidomalonate

15 g of diethyl 4-ethylaminobenzylacetamido-malonate in 70 ml of THF are placed in a three-necked flask which is maintained under a nitrogen atmosphere; 6.4 ml of 2-iodopropane, and then 8.4 ml of 1,5-diazabicyclo[4.3.0]non-5-ene are added and the mixture is then heated at 60°C for 24h. 2.13 ml of 2-iodopropane, and then 8.4 ml of 1,5-diazabicyclo[4.3.0]non-5-ene, are subsequently added and the mixture is then heated for a further 24h at 60°C. The mixture is brought to room temperature and then extracted with 50 ml of dichloromethane and 50 ml of distilled water. The aqueous phase is separated off after settling and then rewashed with 2 times 30 ml of dichloromethane. The organic phases are combined, washed with 60 ml of distilled water and then with 50 ml of distilled water which is saturated with NaCl. The organic phase is separated off after settling, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 16.2 g of a product which is purified by flash chromatography (dichloromethane, MeOH 90/10). This results in 4.59 g of a product which is recrystallized in 45 ml of cyclohexane in order to yield 3.44 g of diethyl 4-ethylisopropylaminobenzylacetamidomalonate in the form of white crystals which melt at 80°C.

39-3: Diethyl 4-ethylaminobenzylacetamidomalonate

Diethyl 4-ethylaminobenzylacetamidomalonate can be prepared by proceeding as in Example 35-7 but using 22 g of diethyl 4-aminobenzylacetamidomalonate, 500 ml of ethanol and 70 g of Raney nickel. This results in 23.8 g of diethyl 4-ethylaminobenzylacetamidomalonate in the form of an off-white solid which melts at 136°C.

39-4: (RS)-4-Allylethylaminophenylalanine dihydrochloride

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 4.54 g of diethyl 4-allylethylbenzylacetamidomalonate and 37.9 ml of 10N hydrochloric acid; the solid is then dried under reduced pressure (2.7 kPa) at 40°C. 3.67 g of (RS)-4-allylethylaminophenylalanine dihydrochloride are obtained in the form of a brown solid which melts in the region of 130°C (decomposition).

39-5: Diethyl 4-allylethylaminobenzylacetamidomalonate

5.6 g of a solid are obtained, after purification by flash chromatography (eluant, CH2Cl2/AcOET 90-10 by volume), by proceeding as in Example 39-2 but using 8 g of diethyl 4-ethylaminobenzylacetamidomalonate, 4 ml of allyl bromide and 5.82 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF; the solid is then recrystallized in 35 ml of cyclohexane.

This results in 5.43 g of diethyl 4-allylethylamino-benzylacetamidomalonate in the form of a white solid which melts at 86°C.

39-6: (RS)-4-Ethylpropylaminophenylalanine dihydrochloride

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.5 g of diethyl 4-ethylpropylaminobenzylacetamidomalonate and 21 ml of 10N hydrochloric acid;. The solid is then dried under reduced pressure (2.7 kPa) at 40°C. 2 g (97%) of (RS)-4-ethylpropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).

39-7: Diethyl 4-ethylpropylaminobenzyl-acetamidomalonate

2.8 g of a solid are obtained, after reacting for 36 hours and then purifying by flash chromatography (eluant, CH_2Cl_2/MeOH 97-3 by volume), by proceeding as in Example 39-2 but using 10 g of diethyl 4-ethylamino-benzylacetamidomalonate, 5.6 ml of 1-iodopropane and 7.2 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 70 ml of THF; the solid is then recrystallized in 26 ml of cyclohexane. This results in 2.9 g of diethyl 4-ethyl-propylaminobenzylacetamidomalonate in the form of a white solid which melts at 84-86°C.

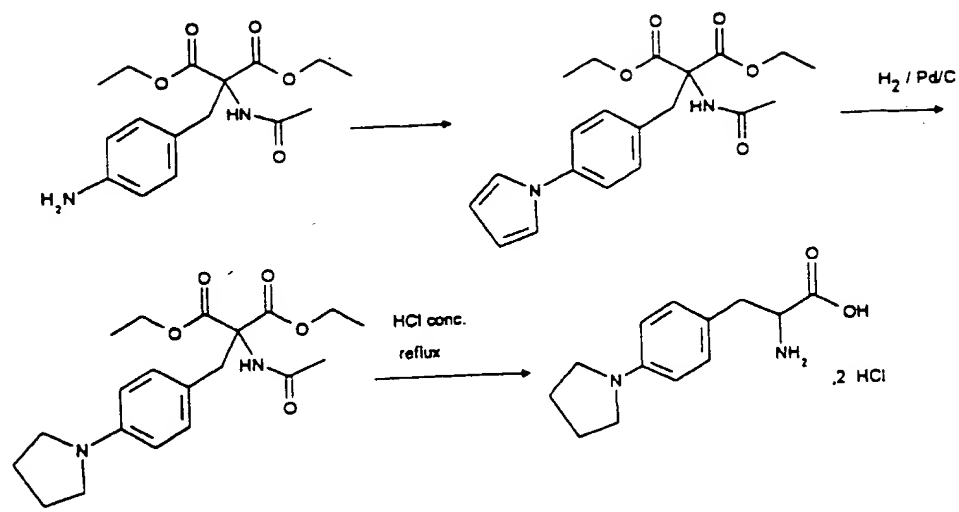
39-8: <u>(RS)-4-Ethylmethylcyclopropylamino-phenylalanine dihydrochloride</u>

A solid is obtained, after reacting for 3 days and then evaporating, by proceeding as in Example 35-1 but using 3 g of diethyl 4-ethylmethylcyclopropyl-aminobenzylacetamidomalonate and 25 ml of 10N hydrochloric acid; the solid is then triturated in 40 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.24 g of (RS)-4-ethylmethylcyclopropylaminophenylalanine dihydro-chloride are obtained in the form of a white solid which melts in the region of 140°C (decomposition).

39-9: <u>Diethyl 4-ethylmethylcyclopropylamino-benzylacetamidomalonate</u>

By proceeding as in Example 39-2, but using 8 g of diethyl 4-ethylaminobenzylacetamidomalonate, 2.6 ml of bromomethylcyclopropane and 2.97 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF, 3.3 g of diethyl 4-ethylmethylcyclopropylaminobenzylacetamido-malonate are obtained, after reacting for 3 days and then purifying by flash chromatography (eluant CH2Cl2/AcOEt 90-10 by volume), in the form of a white solid which melts at 112-114°C.

EXAMPLE 40: Preparation of derivatives of phenylalanine using method G



40-1: <u>(RS)-4-(1-Pyrrolidinyl)phenylalanine dihydrochloride</u>

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 1.5 g of diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate and 40 ml of 5N hydrochloric acid; the solid is then triturated in 15 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 0.6 g of (RS)-4-(1-pyrrolidinyl)phenylanaline dihydrochloride is obtained in the form of an off-white solid.

40-2: <u>Diethyl 4-(1-pyrrolidinyl)benzyl-acetamidomalonate</u>

4 g of d ethyl 4-(1-pyrrolyl)benzylacetamido-malonate, dissolved in 100 ml of MeOH, and 1 g of 10% palladium on charcoal are placed in an autoclave. After having purged the autoclave 3 times with nitrogen, the

product is hydrogenated at 19°C under a pressure of 14 bars of hydrogen. After stirring for 25 hours, the hydrogenation is stopped and the product is filtered through Clarcel® and rinsed with dichloromethane; the solution is then concentrated under reduced pressure in order to yield 3.85 g of a solid which is triturated in a mixture of 50 ml of heptane and 10 ml of ethyl ether. The resulting solid is filtered, dried and then purified by flash chromatography (eluant CH_2Cl_2/acetone 90/10 by volume) in order to yield 1.6 g of diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate in the form of a white solid which melts at 132°C.

40-3: Diethyl 4-(1-pyrrolyl)benzylacetamidomalonate

4,6 g of diethyl 4-aminobenzylacetamidomalonate in 104 ml of acetic acid are placed in a three-necked flask which is maintained under nitrogen. 7.02 g of sodium acetate are added, followed by 1.87 ml of 2,5-dimethoxytetrahydrofuran. The mixture is heated at 65°C for 1h 15 min, then cooled down and extracted with 100 ml of dichloromethane and 100 ml of distilled water. The aqueous phase is separated off after settling and then washed with 3 times 100 ml of dichloromethane. The organic phases are combined, washed with 100 ml of water and then with 100 ml of a saturated solution of NaCl, separated off after settling and then dried over magnesium sulphate; the phases are filtered and then evaporated to dryness

under reduced pressure (50 kPa) in order to yield 6.2 g of a solid which is purifed by flash chromatography (eluent CH2Cl2/acetone 75/25 by volume). This results in 3.57 g of diethyl 4-(1-pyrrolyl)benzylacetamido-malonate in the form of a beige solid which melts at 110°C.

EXAMPLE 41: Preparation of derivatives of phenylalanine using method H

41-1: <u>(RS)-4-Ethylthiomethylphenylalanine</u>

300 ml of anhydrous methanol are placed in a three-necked flask which is maintained under nitrogen; subsequently, 1.72 g of sodium methoxide, and then 5.55 ml of ethyl mercaptan, are added while stirring. The solvent is concentrated under reduced pressure at 40°C in order to yield 8.5 g of the sodium salt of ethyl mercaptan, which is dissolved in 100 ml of anhydrous THF. 3.6 g of (RS)-4-chloromethylphenyl-alanine are added at room temperature and the mixture is then heated to reflux for 18h. The solvent is evaporated under reduced pressure at 40°C and the residue is taken up in 100 ml of distilled water. The turbid solution which is obtained is acidified with 5 ml of acetic acid. The resulting precipitate is filtered, rinsed with distilled water and then dried at 60)C under reduced pressure in order to yield 3.6 g of a solid which is purified by flash chromatography (eluant AcOEt 60, AcOH 12, water 10). This results in

256 mg of (RS)-4-ethylthiomethylphenylalanine in the form of a white solid which melts at 251°C.

The (RS)-4-chloromethylphenylalanine can be obtained by analogy with (S)-4-chloromethylphenylalanine as described in: R.Gonzalez-Muniz, F. Cornille, F. Bergeron, D. Ficheux, J. Pothier, C. Durieux and B. Roques, Int. J. Pept. Protein. Res., 1991, 37 (41), 331-340.

EXAMPLE 42: Preparation of derivatives of phenylalanine using method I

42-1: <u>(S)-4-O-(2-Chloroethyl)tyrosine hydrochloride</u>

5 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine, dissolved in 50 ml of hydrochloric dioxane, are placed in a round-bottomed flask. After having been stirred for 28h, the mixture is concentrated to dryness under reduced pressure. The resulting residue is taken up in 50 ml of ether and this solution is then stirred and filtered. The resulting solid is washed with 2 times 25 ml of ether and then dried under reduced pressure in order to yield 1.58 g of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride in the form of a white solid which melts at 260°C.

42-2: <u>(S)-N-tert-Butoxycarbonyl-4-O-(2-chloroethyl)tyrosine</u>

14 g of (S)-N-tert-butoxycarbonyltyrosine, dissolved in 140 ml of DMF, are placed in a three-

necked flask under a nitrogen atmosphere. 4.8 g of 50% sodium hydride in oil are added slowly using a spatula. 16.87 g of 1-tosyl-2-chloroethanol are added after the mixture has been stirred for 2h at room temperature. 2.4 g of 50% sodium hydride in oil, and a further 8.4 ml of 1-tosyl-2-chloroethanol, are added after the mixture has been stirred for 2 days. The same procedure is carried out after 24h and the stirring is continued for a further 24h. The reaction is stopped by adding 100 ml of distilled water, and the reaction mixture is concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water and then extracted with 3 times 100 ml of ethyl acetate. The aqueous phase is separated off after settling and acidified to pH3 with 50 ml of 1N HCl, and the product is extracted with 3 times 100 ml of ethyl acetate. The organic phases are combined, washed with 2 times 50 ml of water, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 13.51 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine in the form of a chestnut oil (Rf = 0.5, toluene 70%/methanol 20%/diethylamine 10%), which is used as such in the following step.

TABLE V

MICROORGANISMS	ANTIBIOTICS
FUNGI	
Micromonospora sp.	Vernamycins
STREPTOMYCES	
S. alborectus	Virginiamycins
S. conganensis (ATCC13528)	F1370 A, B
S. diastaticus	Plauracins, Streptogramins
S. graminofasciens	Streptogramins
S. griseus (NRRL2426)	Viridogrisein (Etamycin)
S. griseoviridus	Griseoviridin
S. griseoviridus (FERMP3562)	Neoviridogriseins
S. lavendulae	Etamycins
S. loidensis (ATCC11415)	Vernamycins
S. mitakaensis (ATCC15297)	Mikamycins
S. olivaceus (ATCC12019)	Synergistins (PA 114 A, B)
S. ostreogriseus (ATCC27455)	Ostreogrycins
S. pristinaespiralis (ATCC25486)	Pristinamycins
S. virginiae (ATCC13161)	Virginiamycins (Staphylomycins)
ACTINOMYCETES	
A. auranticolor (ATCC31011)	Plauracins
A. azureus (ATCC31157)	Plauracins
A. daghestanicus	Etamycin
A. philippinensis	A-2315 A,B,C
Actinoplanes sp. (ATCC3302)	A15104
Actinoplanes sp.	A17002 A,B,C,F
Actinomadura flava	Madumycins

Abbreviations employed:

AcOEt	ethyl acetate
DNA	deoxyribonucleic acid
AMP	adenosine 5'-monophosphate
HPLC	high-performance liquid chromatography
dCTP	deoxycytosine 5'-triphosphate
DMF	dimethylformamide
DMPAPA	4-dimethylamino-L-phenylalanine
HCl	hydrochloric acid
HT7	Hickey Tresner solid medium
3-HPA	3-hydroxypicolinic acid
IPTG	isopropyl-β-D-thiogalactopyranoside
kb	kilobase
LB	Luria broth (rich growth medium for *E. coli*)
MeOH	methanol
MMPAPA	4-methylamino-L-phenylalanine
NaOH	sodium hydroxide
PAPA	4-amino-L-phenylalanine
PEG	polyethylene glycol
P I	pristinamycin I
P II	pristinamycin II
bp	base pair
SAM	S-adenosylmethionine
TE	10 mM Tris-HCl buffer, 1 mM EDTA, pH 7.5
THF	tetrahydrofuran
Tris	2-amino-2-(hydroxymethyl)-1,3-

	propanediol
UV	ultraviolet rays
X-gal	5-bromo-4-chloro-3-indoyl-β-D-galactoside
YEME	yeast extract-malt extract medium (rich growth medium for Streptomyces)

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: RHONE-POULENC RORER S.A.

(B) STREET: 20, avenue Raymond ARON

(C) CITY: ANTONY

(E) COUNTRY: FRANCE

(F) POSTAL CODE: 92165

(ii) TITLE OF INVENTION: NOVEL STREPTOGRAMINS AND PROCESS FOR PREPARING STREPTOGRAMINS BY MUTASYNTHESIS.

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Tape

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: Release #1.0, Version #1.25 (OEB)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2888 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GCGGACGGCT GGGCGGAGTC CAAACCCATC AAGGGCACCC GCCCCCGCGG CGCCGGCCCC

       130        140        150        160        170        180
GCCCAGGACG CCGCCGTCAA GGCCTCCCTC GCCGCGGCCG AGAAGGACCG CAGCGAGAAC

       190        200        210        220        230        240
CTGATGATCG TCGACCTGGT CCGCAACGAC CTCGGCCAGG TCTGCGACAT CGGCTCCGTC

       250        260        270        280        290        300
CACGTACCGG GCCTGTTCGA GGTGGAGACC TACGCCACCG TCCACCAGCT CGTCAGCACG

       310        320        330        340        350        360
GTCCGCGGCC GCCTGGCGGC CGACGTCTCC CGCCCCCGCG CGGTACGGGC CGCCTTCCCC
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370	380	390	400	410	420
GGCGGGTCGA	TGACCGGCGC	GCCCAAGGTC	CGCACCATGC	AGTTCATCGA	CCGGCTCGAG
430	440	450	460	470	480
AAGGGCCCGC	GCGGCGTGTA	CTCGGGCGCG	CTGGGCTACT	TCGCCCTCAG	CGGCGCGGCC
490	500	510	520	530	540
GACCTCAGCA	TCGTCATCCG	CACCATCGTC	GCCACCGAGG	AGGCCGCCAC	CATCGGCGTG
550	560	570	580	590	600
GGCGGCGCCG	TCGTCGCCCT	GTCCGACCCC	GACGACGAGG	TCCGCGAAAT	GCTCCTCAAG
610	620	630	640	650	660
GCGCAGACCA	CCCTCGCCGC	CCTGCGCCAG	GCACACGCGG	GCGCCACCGC	CTCGGACCGT
670	680	690	700	710	720
GAACTCCTGG	CCGGCAGCCT	GCGGTGACCC	ACCCACCGCC	CCACCCCGGC	CACCGCAACC
730	740	750	760	770	780
CCGGCTCACC	CCCGGGGCGG	CCGCGCGCGG	TGCCGCCCGG	CGGCCGACCC	GGCGACGGGT
790	800	810	820	830	840
CCGCTCGCGG	ACCGGGTGAC	GGACCCGGCG	GCGGGGCCGG	CGGCGGGCCG	GGACGTGGGC
850	860	870	880	890	900
CGGGACGTGG	GCCCGGCGTC	CCCGGCGACC	GGCACGGCGG	CGGGCCCGGA	CGTGGGCCCG
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GCGTGCCCGG	CGACCGGCAC	GGTGGCGGGG	CGGGGCGGGG	GACGGTCAGT	GCAGGGCGGT
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1630	1640	1650	1660	1670	1680
CACCACGTCC	GCCGCCGCCA	GCGCCGCGAC	CGCCTCCGGC	CCCGGCCGCC	GCACATCACC
1690	1700	1710	1720	1730	1740

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1750 1760 1770 1780 1790 1800
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1810 1820 1830 1840 1850 1860
CACCACACAA CGCCCGAACA CCGAACCACC CCTCATCCGC GTTCCCGATC CCCCCGGTAC

1870 1880 1890 1900 1910 1920
GGAGGAAGAA CCATGACCCC GCCCGCCATC CCCGCCGCCC CGCCCGCCAC CGGGCCCGCC

1930 1940 1950 1960 1970 1980
CCCGCCACCG ACCCCCTCGA CGCGCTGCGC GCCCGCCTGG ACGCCGCGGA CGCCGCCCTG

1990 2000 2010 2020 2030 2040
CTGGACGCCG TCCGCACACG CCTGGACATC TGCCTGCGCA TCGGCGAGTA CAAGCGCCTC

2050 2060 2070 2080 2090 2100
CACCAGGTGC CGATGATGCA GCCCCACCGG ATCGCCCAGG TCCACGCCAA CGCCGCCCGC

2110 2120 2130 2140 2150 2160
TACGCCGCCG ACCACGGCAT CGACCCCGCC TTCCTGCGCA CCCTGTACGA CACGATCATC

2170 2180 2190 2200 2210 2220
ACCGAGACCT GCCGCCTCGA GGACGAGTGG ATCGCCTCCG GCGGCGCCCC CGTCCCCACG

2230 2240 2250 2260 2270 2280
CCCGTGCACG CGTCCGCGTC CGCGCGGGGG GCCGTGTCGT GACCGCCGCC GCACCCACCC

2290 2300 2310 2320 2330 2340
TCGCCCAGGC GCTGGACGAG GCCACCGGGC AGCTGACCGG CGCCGGGATC ACCGCCGACG

2350 2360 2370 2380 2390 2400
CCGCCCGGGC CGACACCCGG CTGCTGGCCG CCCACGCCTG CCAGGTCGCC CCGGGGGACC

2410 2420 2430 2440 2450 2460
TCGACACCTG CCTGGCCGGC CCGGTGCCGC CCCGGTTCTG GCACTACGTC CGGCGCCGTC

2470 2480 2490 2500 2510 2520
TGACCCGCGA ACCCGCCGAA CGCATCGTCG GCCACGCCTA CTTCATGGGC CACCGCTTCG

2530 2540 2550 2560 2570 2580
ACCTGGCCCC CGGCGTCTTC GTCCCCAAAC CCGAGACCGA GGAGATCACC CGGGACGCCA

2590 2600 2610 2620 2630 2640
TCGCCCGCCT GGAGGCCCTC GTCCGCCGCG GCACCACCGC ACCCCTGGTC GTCGACCTGT

2650 2660 2670 2680 2690 2700
GCGCCGGACC GGGCACCATG GCCGTCACCC TGGCCCGCCA CGTACCGGCC GCCCGCGTCC

2710 2720 2730 2740 2750 2760
TGGGCATCGA ACTCTCCCAG GCCGCCGCCC GCGCCGCCCG GCGCAACGCC CGCGGCACCG

2770 2780 2790 2800 2810 2820
GCGCCCGCAT CGTGCAGGGC GACGCCCGCG ACGCCTTCCC CGAACTGAGC GGCACCGTCG

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TGCTCGAG

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 888 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iv) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

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ATG AGG GGT GGT TCG GTG TTC GGG CGT TGT GTG GTG GTG GGC GGG GCC GGT GCG   54
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GTG GGC CGC ATG TTC AGC CAC TGG CTG GTG CGT TCG GGG GTG GCG GTG ACC TGG  108
Val Gly Arg Met Phe Ser His Trp Leu Val Arg Ser Gly Val Ala Val Thr Trp   36

CTG GAC GTG GCC GGG GCC GGT GCG GCG GAC GGG GTG CGG GTG GTG GCC GGT GAT  162
Leu Asp Val Ala Gly Ala Gly Ala Ala Asp Gly Val Arg Val Val Ala Gly Asp   54

GTG CGG CGG CCG GGG CCG GAG GCG GTC GCG GCG CTG GCG GCG GCG GAC GTG GTG  216
Val Arg Arg Pro Gly Pro Glu Ala Val Ala Ala Leu Ala Ala Ala Asp Val Val   72

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Val Leu Ala Val Pro Glu Pro Val Ala Trp Glu Ala Val Glu Val Leu Ala Gly   90

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Val Met Arg Pro Gly Ala Val Leu Ala Asp Thr Leu Ser Val Lys Ser Arg Ile  108

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Thr Asp Gly Pro Gly Val Arg Ala Leu Val Glu Leu Val Ala Gly Trp Gly Ala  162

CGG GTG GTG GAG ATG CCG GCG CGG CGG CAC GAC GAG CTG ACC GCC GCG CAG CAG  540
Arg Val Val Glu Met Pro Ala Arg Arg His Asp Glu Leu Thr Ala Ala Gln Gln  180

GCC GCC ACG CAT GCC GCG GTG CTG GCC TTC GGG CTG GGC CTG GGT GAG CTG TCG  594
Ala Ala Thr His Ala Ala Val Leu Ala Phe Gly Leu Gly Leu Gly Glu Leu Ser  198
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Val Asp Val Gly Ala Leu Arg Asp Ser Ala Pro Pro Pro His Leu Ala Met Leu 216

GCG CTG CTG GCC CGG ATC GCC GGC GGG ACG CCG GAG GTG TAT TTC GAC ATC CAG 702
Ala Leu Leu Ala Arg Ile Ala Gly Gly Thr Pro Glu Val Tyr Phe Asp Ile Gln 234

GCC GCC AAC CCC GGC GCG CCG GCC GCG CGG CAG GCG CTG GGC CGC GGC CTG GTG 756
Ala Ala Asn Pro Gly Ala Pro Ala Ala Arg Gln Ala Leu Gly Arg Gly Leu Val 252

CGG CTG GGG CAG GCC GTC GAG AGG GGC GAC GAG GAG ACG TTC GCC GCC CTG TTC 810
Arg Leu Gly Gln Ala Val Glu Arg Gly Asp Glu Glu Thr Phe Ala Ala Leu Phe 270

GCC GAA CTG CGC GGT GTG CTG GGC GAG CAC GGC GCG GAG CTG GAA CGG CTG TGC 864
Ala Glu Leu Arg Gly Val Leu Gly Glu His Gly Ala Glu Leu Glu Arg Leu Cys 288

GCG CGG ATG TTC ACC GCC CTG CAC 888
Ala Arg Met Phe Thr Ala Leu His 296

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 387 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

ATG ACC CCG CCC GCC ATC CCC GCC GCC CCG CCC GCC ACC GGG CCC GCC CCC GCC 54
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ACC GAC CCC CTC GAC GCG CTG CGC GCC CGC CTG GAC GCC GCG GAC GCC GCC CTG 108
Thr Asp Pro Leu Asp Ala Leu Arg Ala Arg Leu Asp Ala Ala Asp Ala Ala Leu 36

CTG GAC GCC GTC CGC ACA CGC CTG GAC ATC TGC CTG CGC ATC GGC GAG TAC AAG 162
Leu Asp Ala Val Arg Thr Arg Leu Asp Ile Cys Leu Arg Ile Gly Glu Tyr Lys 54

CGC CTC CAC CAG GTG CCG ATG ATG CAG CCC CAC CGG ATC GCC CAG GTC CAC GCC 216
Arg Leu His Gln Val Pro Met Met Gln Pro His Arg Ile Ala Gln Val His Ala 72

AAC GCC GCC CGC TAC GCC GCC GAC CAC GGC ATC GAC CCC GCC TTC CTG CGC ACC 270
Asn Ala Ala Arg Tyr Ala Ala Asp His Gly Ile Asp Pro Ala Phe Leu Arg Thr 90

CTG TAC GAC ACG ATC ATC ACC GAG ACC TGC CGC CTC GAG GAC GAG TGG ATC GCC 324
Leu Tyr Asp Thr Ile Ile Thr Glu Thr Cys Arg Leu Glu Asp Glu Trp Ile Ala 108

TCC GGC GGC GCC CCC GTC CCC ACG CCC GTG CAC GCG TCC GCG TCC GCG CGG GGG 378
Ser Gly Gly Ala Pro Val Pro Thr Pro Val His Ala Ser Ala Ser Ala Arg Gly 126

GCC GTG TCG 387
Ala Val Ser 129

INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4496 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

10	20	30	40	50	60
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GCCCAGACGG	AACAGCGCGA	AGCACACCGC	GACGAACTCG	GGCGTGTTCG	GCAGCTGCAC
130	140	150	160	170	180
CAGCACCCGC	TCGCCGGCGC	CGATCCCGCG	CGCCGCGAAC	CCCGCCGCCA	GCCGGTCGCA
190	200	210	220	230	240
CCAGCGGTCC	AGGGCACGGT	AGGTGACACG	GGAGCACCCG	TCCGCGCCGA	CCAGCGCCTC
250	260	270	280	290	300
CCGCTCGCCG	TACTGCTCCG	CCCAGCGGCC	CAGCAGCATG	CCCAGCGGCT	CGCCCCGCCA
310	320	330	340	350	360
GTAGCCGGCC	GCCCGGTACT	TCGCGGCCAC	ATCCTCGGGC	CAGGGAACGC	ATCCGTCCAG
370	380	390	400	410	420

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430 440 450 460 470 480
CACGCCGCTG ATGCGCCGCG CCCGCGCGCC GCCGCTCCGT CAGGAGCCGA TCAGGGCGGC

490 500 510 520 530 540
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CACCCGATGG AGACCTGGGT CCTGGGCCGG CGCGACGTCG CCGAGGTGGT GGCCGCCGTC

670 680 690 700 710 720
GGCCGCGACG AACTCATGCG CCGCATCATC GACCGCCTCA CCGGCGGACT GGCCGAGATC

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790 800 810 820 830 840
CCCGGCATCT GGGAATGGAT GCCGCACCGC GAACCCGGCG ACCACATCAC CCTCAAGACC

850 860 870 880 890 900
GTCGGCTACA GCCCCGCCAA CCCCGGCCGC TTCGGCCTGC CGACCATCCT GGGCACCGTC

910 920 930 940 950 960
GCCCGCTACG ACGACACCAC CGGCGCCCTG ACCGCCCTGA TGGACGGCGT GCTGCTCACC

970 980 990 1000 1010 1020
GCCCTGCGCA CCGGCGCCGC CTCCGCCGTC GCCTCCCGCC TGCTGGCCCG CCCCGACAGC

1030 1040 1050 1060 1070 1080
CACACCCTGG GACTGATCGG CACCGGCGCC CAGGCCGTCA CCCAACTGCA CGCCCTGTCC

1090 1100 1110 1120 1130 1140
CTGGTACTGC CCCTGCAACG GGCCCTGGTG TGGGACACCG ACCCCGCCCA CCGGGAAAGC

1150 1160 1170 1180 1190 1200
TTCGCCCGGC GCGCCGCGTT CACCGGCGTC AGCGTCGAGA TCGCCGAGCC CGCCCGGATC

1210 1220 1230 1240 1250 1260
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1270 1280 1290 1300 1310 1320
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1330 1340 1350 1360 1370 1380
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1390 1400 1410 1420 1430 1440
GAGCAGGCGC TGCGCGAGGG CGAGTGCCAG CAACTCTCCG CCGACCGGCT CGGCCCGCAG

1450 1460 1470 1480 1490 1500
CTGGCCCACC TGTGCGCCGA CCCGGCGGCC GCCGCCGGCC GGCAGGACAC CCTGAGCGTC

1510 1520 1530 1540 1550 1560
TTCGACTCCA CCGGCTTCGC CTTCGAGGAC GCCCTGGCGA TGGAAGTGTT CCTCGAGGCC

1570 1580 1590 1600 1610 1620
GCCGCCGAAC GGGACCTGGG CATCCGGGTG GGCATCGAAC ACCACCCCGG CGACGCCCTG

1630 1640 1650 1660 1670 1680
GACCCCTACG CCCTCCAGCC CCTGCCCCTG CCCCTGGCCG CCCCCGCCCA CTGACCCCCC

1690 1700 1710 1720 1730 1740
CCTTTTTTCG GGACCCCCGC TCTTTTTCGA GA[illegible]CCCCGCC CGGCCGGCCC GGCCCTCCTC

1750 1760 1770 1780 1790 1800
CCGCCGGCCC CCATGCCCGG CCGGGCCGGG GCACCCACGA CGCCCTCGCG AGGAGAGAGA

1810 1820 1830 1840 1850 1860
TGCCCCCCAC CCCCCGGCCC ACCACCGACG ACGGCGGCCG TGAACTGCTC GCCTGGCTGC

1870 1880 1890 1900 1910 1920
GCGAGATGCG CCACCACCAC CCCGTCCACG AGGACGAATA CGGTGCCTTC CACGTCTTCC

1930 1940 1950 1960 1970 1980
GGCACGCCGA CGTCCTCACC GTCGCCTCCG ACCCCGGCGT CTACTCCTCC CAGCTCAGCC

1990 2000 2010 2020 2030 2040
GGCTACGGCC CGGCTCCCAG GCGTTGAGCG AACAGATCCT GTCGGTCATC GACCCGCCGA

2050 2060 2070 2080 2090 2100
TGCACCGCAC CCTGCGCCGC CTGGTCAGCC AGGCCTTCAC CCCCCGCACC GTCGCCGACC

2110 2120 2130 2140 2150 2160
TCGAACCACG CGTCACCGAA CTGGCCGGGC AACTGCTCGA CGCCGTCGAC GGCGACACGT

2170 2180 2190 2200 2210 2220
TCGACCTCGT CGCCGACTTC GCCTACCCGC TGCCCGTGAT CGTGATCGCC GAACTCCTCG

2230 2240 2250 2260 2270 2280
GCGTGCCGCC CGCCGACCGC ACCCTGTTCC GCTCCTGGTC CGACCGGATG CTGCAGATGC

2290 2300 2310 2320 2330 2340
AGGTCGCCGA CCCGGCGGAC ATGCAGTTCG GCGACGACGC CGACGAGGAC TACCAACGCC

2350 2360 2370 2380 2390 2400
TCGTCAAAGA ACCCATGCGC GCCATGCACG CCTACCTCCA CGACCACGTC ACCGACCGCC

2410 2420 2430 2440 2450 2460
GCGCCCGCCC CGCGAACGAC CTGATCTCCG CACTCGTCGC CGCCCGCGTG GAGGGCGAAC

2470 2480 2490 2500 2510 2520
GACTCACCGA CGAGCAGATC GTCGAATTCG GGGCGCTGCT GCTGATGGCC GGCCACGTCT

2530 2540 2550 2560 2570 2580
CCACCTCCAT GCTGCTCGGC AACACCGTGC TGTGCCTGAA GGACCACCCC CGGGCCGAGG

2590 2600 2610 2620 2630 2640
CCGCCGCCCG CGCCGACCGG TCCCTGATCC CCGCCCTGAT CGAAGAAGTA CTGCGGCTGC

2650 2660 2670 2680 2690 2700
GGCCGCCGAT CACCGTCATG GCCCGCGTCA CCACCAAGGA CACCGTCCTC GCCGGCACCA

2710 2720 2730 2740 2750 2760
CCATCCCCGC CGGACGCATG GTCGTGCCCT CCCTGCTGTC CGCCAACCAC GACGAACAGG

2770 2780 2790 2800 2810 2820
TCTTCACCGA CCCCGACCAC CTCGACCTCG CCCGCGAAGG CCGCCAGATC GCCTTCGGCC

2830 2840 2850 2860 2870 2880
ACGGCATCCA CTACTGCCTG GGCGCCCCGC TCGCCCGCCT GGAGGGCCGC ATCGCCCTGG

2890 2900 2910 2920 2930 2940
AAGCCCTCTT CGACCGATTC CCCGACTTCT CGCCCACCGA CGGCGCAAAA CTGCGCTACC

2950 2960 2970 2980 2990 3000
ACCGCGACGG ACTGTTCGGC GTCAAGAACC TGCCGCTGAC CGTACGGCGC GGCTGACACA

3010 3020 3030 3040 3050 3060
GACAAGGGGG CCACCTGGTG CGCACCGTGC GAACCCTGCT GATCGACAAC TACGACTCGT

3070 3080 3090 3100 3110 3120
TCACCTACAA CCTCTTCCAG ATGCTGGCCG AGGTGAACGG CGCCGCTCCG CTCGTCGTCC

3130 3140 3150 3160 3170 3180
GCAACGACGA CACCCGCACC TGGCAGGCCC TGGCGCCGGG CGACTTCGAC AACGTCGTCG

3190 3200 3210 3220 3230 3240
TCTCACCCGG CCCCGGCCAC CCCGCCACCG ACACCGACCT GGGCCTCAGC CGCCGGGTGA

3250 3260 3270 3280 3290 3300
TCACCGAATG GGACCTGCCG CTGCTCGGGG TGTGCCTGGG CCACCAGGCC CTGTGCCTGC

3310 3320 3330 3340 3350 3360
TCGCCGGCGC CGCCGTCGTC CACGCACCCG AACCCTTTCA CGGCCGCACC AGCGACATCC

3370 3380 3390 3400 3410 3420
GCCACGACGG GCAGGGCCTG TTCGCGAACA TCCCCTCCCC GCTGACCGTG GTCCGCTACC

3430 3440 3450 3460 3470 3480
ACTCGCTGAC CGTCCGGCAA CTGCCCGCCG ACCTGCGCGC CACCGCCCAC ACCGCCGACG

3490 3500 3510 3520 3530 3540
GGCAGCTGAT GGCCGTCGCC CACCGCCACC TGCCCCGCTT CGGCGTGCAG TTCCACCCCG

3550 3560 3570 3580 3590 3600
AATCGATCAG CAGCGAACAC GGCCACCGGA TGCTCGCCAA CTTCCGCGAC CTGTCCCTGC

3610 3620 3630 3640 3650 3660
GCGCGGCCGG CCACCGCCCC CCGCACACCG AACGCATACC CGCACCCGCA CCCGCCCCCG

3670 3680 3690 3700 3710 3720
CCCCCGCCCC CGCACCGGCA CCGCCCGCGT CCGCGCCGGT GGGGGAGTAC CGGCTGCATG

3730 3740 3750 3760 3770 3780
TGCGCGAGGT CGCCTGCGTG CCCGACGCGG ACGCCGCGTT CACCGCCCTG TTCGCCGACG

3790 3800 3810 3820 3830 3840
CCCCGGCCCG GTTCTGGCTC GACAGCAGCC GCGTCGAGCC GGGCCTCGCC CGCTTCACCT

3850 3860 3870 3880 3890 3900
TCCTCGGCGC CCCCGCCGGC CCGCTCGGCG AACAGATCAC CTACGACGTC GCCGACCGGG

3910 3920 3930 3940 3950 3960
CCGTGCGCGT CAAGGACGGT TCAGGCGGCG AGACCCGCCG GCCCGGCACC CTCTTCGACC

3970 3980 3990 4000 4010 4020
ACCTGGAACA CGAACTGGCC GCCCGCGCCC TGCCCGCCAC CGGCCTGCCC TTCGAGTTCA

4030 4040 4050 4060 4070 4080
ACCTCGGCTA CGTCGGCTAC CTCGGCTACG AGACCAAGGC CGACAGCGGC GGCGAGGACG

4090 4100 4110 4120 4130 4140
CCCACCGCGG CGAACTGCCC GACGGCGCCT TCATGTTCGC CGACCGGATG CTCGCCCTCG

4150 4160 4170 4180 4190 4200
ACCACGAACA GGGGCGGGCC TGGCTCCTGG CACTGAGCAG CACCCGACGG CCCGCCACCG

4210 4220 4230 4240 4250 4260
CACCCGCCGC CGAACGCTGG CTCACCGACG CCGCCCGGAC CCTCGCCACC ACCGCCCCCC

4270 4280 4290 4300 4310 4320
GCCCGCCCTT CACCCTGCTG CCCGACGACC AACTGCCCGC CCTGGACGTC CACTACCGCC

4330 4340 4350 4360 4370 4380
ACAGCCTGCC CCGCTACCGG GAACTGGTCG AGGAATGCCG CCGCCTGATC ACCGACGGCG

4390 4400 4410 4420 4430 4440

AGACCTACGA GGTGTGCCTG ACGAACATGC TCCGGGTGCC CGGCCGGATC GACCCGCTCA

4450 4460 4470 4480 4490
CCGCCTACCG CGCCCTGCGC ACCGTCAGCC CCGCCCCCTA CGCCGCCTAC CTGCAG

(6) INFORMATION FOR SEQ ID NO: 5

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1065 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptomyces pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

```
ATG GAG ACC TGG GTC CTG GGC CGG CGC GAC GTC GCC GAG GTG GTG GCC GCC GTC     54
Met Glu Thr Trp Val Leu Gly Arg Arg Asp Val Ala Glu Val Val Ala Ala Val     18

GGC CGC GAC GAA CTC ATG CGC CGC ATC ATC GAC CGC CTC ACC GGC GGA CTG GCC    108
Gly Arg Asp Glu Leu Met Arg Arg Ile Ile Asp Arg Leu Thr Gly Gly Leu Ala     36

GAG ATC GGC CGC GGC GAG CGG CAC CTG TCC CCG CTG CGC GGC GGA CTG GAA CGC    162
Glu Ile Gly Arg Gly Glu Arg His Leu Ser Pro Leu Arg Gly Gly Leu Glu Arg     54

AGC GAA CCC GTG CCC GGC ATC TGG GAA TGG ATG CCG CAC CGC GAA CCC GGC GAC    216
Ser Glu Pro Val Pro Gly Ile Trp Glu Trp Met Pro His Arg Glu Pro Gly Asp     72

CAC ATC ACC CTC AAG ACC GTC GGC TAC AGC CCC GCC AAC CCC GGC CGC TTC GGC    270
His Ile Thr Leu Lys Thr Val Gly Tyr Ser Pro Ala Asn Pro Gly Arg Phe Gly     90

CTG CCG ACC ATC CTG GGC ACC GTC GCC CGC TAC GAC GAC ACC ACC GGC GCC CTG    324
Leu Pro Thr Ile Leu Gly Thr Val Ala Arg Tyr Asp Asp Thr Thr Gly Ala Leu    108

ACC GCC CTG ATG GAC GGC GTG CTG CTC ACC GCC CTG CGC ACC GGC GCC GCC TCC    378
Thr Ala Leu Met Asp Gly Val Leu Leu Thr Ala Leu Arg Thr Gly Ala Ala Ser    126

GCC GTC GCC TCC CGC CTG CTG GCC CGC CCC GAC AGC CAC ACC CTG GGA CTG ATC    432
Ala Val Ala Ser Arg Leu Leu Ala Arg Pro Asp Ser His Thr Leu Gly Leu Ile    144

GGC ACC GGC GCC CAG GCC GTC ACC CAA CTG CAC GCC CTG TCC CTG GTA CTG CCC    486
Gly Thr Gly Ala Gln Ala Val Thr Gln Leu His Ala Leu Ser Leu Val Leu Pro    162
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CTG CAA CGG GCC CTG GTG TGG GAC ACC GAC CCC GCC CAC CGG GAA AGC TTC GCC 540
Leu Gln Arg Ala Leu Val Trp Asp Thr Asp Pro Ala His Arg Glu Ser Phe Ala 180

CGG CGC GCC GCG TTC ACC GGC GTC AGC GTC GAG ATC GCC GAG CCC GCC CGG ATC 594
Arg Arg Ala Ala Phe Thr Gly Val Ser Val Glu Ile Ala Glu Pro Ala Arg Ile 198

GCC GCC GAG GCC GAC GTC ATC TCC ACC GCC ACC TCG GTA GCC GTC GGC CAG GGC 648
Ala Ala Glu Ala Asp Val Ile Ser Thr Ala Thr Ser Val Ala Val Gly Gln Gly 216

CCG GTC CTG CCC GAC ACC GGC GTC CGC GAG CAC CTG CAC ATC AAC GCC GTC GGC 702
Pro Val Leu Pro Asp Thr Gly Val Arg Glu His Leu His Ile Asn Ala Val Gly 234

GCG GAC CTC GTC GGC AAG ACG GAA CTG CCG CTC GGC CTG CTC GAG CGG GCG TTC 756
Ala Asp Leu Val Gly Lys Thr Glu Leu Pro Leu Gly Leu Leu Glu Arg Ala Phe 252

GTC ACC GCC GAC CAC CCC GAG CAG GCG CTG CGC GAG GGC GAG TGC CAG CAA CTC 810
Val Thr Ala Asp His Pro Glu Gln Ala Leu Arg Glu Gly Glu Cys Gln Gln Leu 270

TCC GCC GAC CGG CTC GGC CCG CAG CTG GCC CAC CTG TGC GCC GAC CCG GCG GCC 864
Ser Ala Asp Arg Leu Gly Pro Gln Leu Ala His Leu Cys Ala Asp Pro Ala Ala 288

GCC GCC GGC CGG CAG GAC ACC CTG AGC GTC TTC GAC TCC ACC GGC TTC GCC TTC 918
Ala Ala Gly Arg Gln Asp Thr Leu Ser Val Phe Asp Ser Thr Gly Phe Ala Phe 306

GAG GAC GCC CTG GCG ATG GAA GTG TTC CTC GAG GCC GCC GCC GAA CGG GAC CTG 972
Glu Asp Ala Leu Ala Met Glu Val Phe Leu Glu Ala Ala Ala Glu Arg Asp Leu 324

GGC ATC CGG GTG GGC ATC GAA CAC CAC CCC GGC GAC GCC CTG GAC CCC TAC GCC 1026
Gly Ile Arg Val Gly Ile Glu His His Pro Gly Asp Ala Leu Asp Pro Tyr Ala 342

CTC CAG CCC CTG CCC CTG CCC CTG GCC GCC CCC GCC CAC 1065
Leu Gln Pro Leu Pro Leu Pro Leu Ala Ala Pro Ala His 355

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1194 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyc s pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

```
ATG CCC CCC ACC CCC CGG CCC ACC ACC GAC GAC GGC GGC CGT GAA CTG CTC GCC   54
Met Pro Pro Thr Pro Arg Pro Thr Thr Asp Asp Gly Gly Arg Glu Leu Leu Ala   18

TGG CTG CGC GAG ATG CGC CAC CAC CAC CCC GTC CAC GAG GAC GAA TAC GGT GCC  108
Trp Leu Arg Glu Met Arg His His His Pro Val His Glu Asp Glu Tyr Gly Ala   36

TTC CAC GTC TTC CGG CAC GCC GAC GTC CTC ACC GTC GCC TCC GAC CCC GGC GTC  162
Phe His Val Phe Arg His Ala Asp Val Leu Thr Val Ala Ser Asp Pro Gly Val   54

TAC TCC TCC CAG CTC AGC CGG CTA CGG CCC GGC TCC CAG GCG TTG AGC GAA CAG  216
Tyr Ser Ser Gln Leu Ser Arg Leu Arg Pro Gly Ser Gln Ala Leu Ser Glu Gln   72

ATC CTG TCG GTC ATC GAC CCG CCG ATG CAC CGC ACC CTG CGC CGC CTG GTC AGC  270
Ile Leu Ser Val Ile Asp Pro Pro Met His Arg Thr Leu Arg Arg Leu Val Ser   90

CAG GCC TTC ACC CCC CGC ACC GTC GCC GAC CTC GAA CCA CGC GTC ACC GAA CTG  324
Gln Ala Phe Thr Pro Arg Thr Val Ala Asp Leu Glu Pro Arg Val Thr Glu Leu  108

GCC GGG CAA CTG CTC GAC GCC GTC GAC GGC GAC ACG TTC GAC CTC GTC GCC GAC  378
Ala Gly Gln Leu Leu Asp Ala Val Asp Gly Asp Thr Phe Asp Leu Val Ala Asp  126

TTC GCC TAC CCG CTG CCC GTG ATC GTG ATC GCC GAA CTC CTC GGC GTG CCG CCC  432
Phe Ala Tyr Pro Leu Pro Val Ile Val Ile Ala Glu Leu Leu Gly Val Pro Pro  144

GCC GAC CGC ACC CTG TTC CGC TCC TGG TCC GAC CGG ATG CTG CAG ATG CAG GTC  486
Ala Asp Arg Thr Leu Phe Arg Ser Trp Ser Asp Arg Met Leu Gln Met Gln Val  162

GCC GAC CCG GCG GAC ATG CAG TTC GGC GAC GAC GCC GAC GAG GAC TAC CAA CGC  540
Ala Asp Pro Ala Asp Met Gln Phe Gly Asp Asp Ala Asp Glu Asp Tyr Gln Arg  180

CTC GTC AAA GAA CCC ATG CGC GCC ATG CAC GCC TAC CTC CAC GAC CAC GTC ACC  594
Leu Val Lys Glu Pro Met Arg Ala Met His Ala Tyr Leu His Asp His Val Thr  198

GAC CGC CGC GCC CGC CCC GCG AAC GAC CTG ATC TCC GCA CTC GTC GCC GCC CGC  648
Asp Arg Arg Ala Arg Pro Ala Asn Asp Leu Ile Ser Ala Leu Val Ala Ala Arg  216

GTG GAG GGC GAA CGA CTC ACC GAC GAG CAG ATC GTC GAA TTC GGG GCG CTG CTG  702
Val Glu Gly Glu Arg Leu Thr Asp Glu Gln Ile Val Glu Phe Gly Ala Leu Leu  234

CTG ATG GCC GGC CAC GTC TCC ACC TCC ATG CTG CTC GGC AAC ACC GTG CTG TGC  756
Leu Met Ala Gly His Val Ser Thr Ser Met Leu Leu Gly Asn Thr Val Leu Cys  252

CTG AAG GAC CAC CCC CGG GCC GAG GCC GCC GCC CGC GCC GAC CGG TCC CTG ATC  810
Leu Lys Asp His Pro Arg Ala Glu Ala Ala Ala Arg Ala Asp Arg Ser Leu Ile  270

CCC GCC CTG ATC GAA GAA GTA CTG CGC CTG CGG CCG CCG ATC ACC GTC ATG GCC  864
Pro Ala Leu Ile Glu Glu Val Leu Arg Leu Arg Pro Pro Ile Thr Val Met Ala  288
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CGC GTC ACC ACC AAG GAC ACC GTC CTC GCC GGC ACC ACC ATC CCC GCC GGA CGC 918
Arg Val Thr Thr Lys Asp Thr Val Leu Ala Gly Thr Thr Ile Pro Ala Gly Arg 306

ATG GTC GTG CCC TCC CTG CTG TCC GCC AAC CAC GAC GAA CAG GTC TTC ACC GAC 972
Met Val Val Pro Ser Leu Leu Ser Ala Asn His Asp Glu Gln Val Phe Thr Asp 324

CCC GAC CAC CTC GAC CTC GCC CGC GAA GGC CGC CAG ATC GCC TTC GGC CAC GGC 1026
Pro Asp His Leu Asp Leu Ala Arg Glu Gly Arg Gln Ile Ala Phe Gly His Gly 342

ATC CAC TAC TGC CTG GGC GCC CCG CTC GCC CGC CTG GAG GGC CGC ATC GCC CTG 1080
Ile His Tyr Cys Leu Gly Ala Pro Leu Ala Arg Leu Glu Gly Arg Ile Ala Leu 360

GAA GCC CTC TTC GAC CGA TTC CCC GAC TTC TCG CCC ACC GAC GGC GCA AAA CTG 1134
Glu Ala Leu Phe Asp Arg Phe Pro Asp Phe Ser Pro Thr Asp Gly Ala Lys Leu 378

CGC TAC CAC CGC GAC GGA CTG TTC GGC GTC AAG AAC CTG CCG CTG ACC GTA CGG 1188
Arg Tyr His Arg Asp Gly Leu Phe Gly Val Lys Asn Leu Pro Leu Thr Val Arg 396

CGC GGC 1194
Arg Gly 398

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1561 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

10	20	30	40	50	60
AAGCTTCCCG	ACCGGGTGGA	GGTCGTCGAC	GCGTTCCCGC	TGACCGGCCT	CAACAAGGTC
70	80	90	100	110	120
GACAAGAAGG	CCCTGGCGGC	CGACATCGCC	GCCAAGACCG	CCCCCACCCG	CCCCACCACC
130	140	150	160	170	180
GCCGGCCACG	GCCCGACCAC	GGACGGCGAT	ACGGCCGGTG	GGGGTGGGTC	CGCGGGCGGG
190	200	210	220	230	240
GTGACGGCCG	CCGGTGGCGC	GCGGGAGGAG	GCGGCGTGAG	CGGGCCCGGG	CCCGAGGGCG
250	260	270	280	290	300
GCTACCGGGT	GCCGTTCGCG	CGACGCGGTT	CGGTGGTGGG	CGAGGCGGAC	CTGGCGGCGC

310	320	330	340	350	360
TGGGCGAACT	GGTCCGCTCG	GGCCGGTCGC	TGACGTCGGG	GGTGTGGCGG	GAGCGGTTCG
370	380	390	400	410	420
AGGAACAGTT	CGCCCGCCTG	ACCGGCGCCC	GGCACGCGCT	CAGTGTCACC	AGCGGCACCG
430	440	450	460	470	480
TCGCGCTGGA	ACTGGCGGTG	CGGATGCTGG	ACCTGGCGCC	GGGCGACGAG	GTGATCGCCA
490	500	510	520	530	540
CCCCGCAGAC	GTTCCAGGCG	ACGGTGCAGC	CGCTGCTCGA	CCACGACGTG	CGGCTGCGGT
550	560	570	580	590	600
TCTGCGACAT	CGACCCGGAC	ACCCTCAACC	TCGACCCGGC	GGTGCTGGAG	ACGCTGATCA
610	620	630	640	650	660
CCGACCGCAC	CCGGGCGATC	CTGCTCGTCC	ACTACGGCGG	CAACCCGGCC	GACATGGACC
670	680	690	700	710	720
GCATCATGGC	CCTGGCCCGC	AAGCGCGGCA	TCATCGTCGT	CGAGGACAGC	GCGCACGCGC
730	740	750	760	770	780
TGGGCGCCGT	GTACCGGGGG	CGGCGGCCGG	GGGCACTGGC	GGACATCGGC	TGCTTCACTT
790	800	810	820	830	840
TCCACTCCAC	GAAGAACATC	ACCACCCTCG	GCGAGGGCGG	CATGATCACC	CTGTCGCGTG
850	860	870	880	890	900
ACGAGTGGGC	CCAGCGGGTG	GGACGTATCC	GCGACAACGA	GGCCGACGGC	GTGTACGCGG
910	920	930	940	950	960
CGCTGCCGGA	CTCCGCGCGG	GCGGGTGCTC	CGGCGCTGCT	GCCGTGGATG	AAGTTCGCGG
970	980	990	1000	1010	1020
AGGGTGTGTA	CGGTCACCGG	GCGGTCGGGG	TCCGCGGGGC	GGGCACGAAC	GCGACGATGT
1030	1040	1050	1060	1070	1080
CGGAGGCGGC	GGCGGCGGTG	GGCGTGGTGC	AACTGGCGTC	GCTGGAGCGG	TTCGTGGCCC
1090	1100	1110	1120	1130	1140
GGCGCCGGAG	CATCGCGCAG	CGGCTGGACG	AGGCCGTGGC	CTCGGTGGCC	GGCACCCGGC
1150	1160	1170	1180	1190	1200
TGCACCGGGC	GGCGGCGGAC	AGTCTGCACG	CCTACCACCT	GTACACGTTC	TTCCTCACCG
1210	1220	1230	1240	1250	1260
GCGGCCGGCA	GGTGCGGGAG	CGGTTCGTGC	GCGCCCTGGA	CCGGCTGGGT	GTGGAGGTCC
1270	1280	1290	1300	1310	1320
AGTTGCGGTA	CTTCCCGCTC	CATCTGTCGC	CCGAGTGGCG	GCTGCGCGGC	CACGGGCCGG
1330	1340	1350	1360	1370	1380
GCGAGTGTCC	GACGGCCGAA	CGGGTCTGGT	TCGAGGAGCA	CATGAACCTG	CCGTGCCATC
1390	1400	1410	1420	1430	1440
CCGGTCTGAG	TGACGGCCAG	GTCGACTACA	TGGTCGAGGC	GGTCACCCGC	GCCCTGCACG
1450	1460	1470	1480	1490	1500
AGGCCCACGG	CACGGGGACG	CGGGTGGCGG	CCGGGCACCT	GTGACACCGT	CCGCATCCGG
1510	1520	1530	1540	1550	1560
CCGGTGGTTT	TCCAAGACCG	AGGGAGAGGC	AGGCGTATGC	CGTTCATCGA	AGTGAAGATC

T

(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1233 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8

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GTG CCG TTC GCG CGA CGC GGT TCG GTG GTG GGC GAG GCG GAC CTG GCG GCG CTG    54
Val Pro Phe Ala Arg Arg Gly Ser Val Val Gly Glu Ala Asp Leu Ala Ala Leu    18

GGC GAA CTG GTC CGC TCG GGC CGG TCG CTG ACG TCG GGG GTG TGG CGG GAG CGG   108
Gly Glu Leu Val Arg Ser Gly Arg Ser Leu Thr Ser Gly Val Trp Arg Glu Arg    36

TTC GAG GAA CAG TTC GCC CGC CTG ACC GGC GCC CGG CAC GCG CTC AGT GTC ACC   162
Phe Glu Glu Gln Phe Ala Arg Leu Thr Gly Ala Arg His Ala Leu Ser Val Thr    54

AGC GGC ACC GTC GCG CTG GAA CTG GCG GTG CGG ATG CTG GAC CTG GCG CCG GGC   216
Ser Gly Thr Val Ala Leu Glu Leu Ala Val Arg Met Leu Asp Leu Ala Pro Gly    72

GAC GAG GTG ATC GCC ACC CCG CAG ACG TTC CAG GCG ACG GTG CAG CCG CTG CTC   270
Asp Glu Val Ile Ala Thr Pro Gln Thr Phe Gln Ala Thr Val Gln Pro Leu Leu    90

GAC CAC GAC GTG CGG CTG CGG TTC TGC GAC ATC GAC CCG GAC ACC CTC AAC CTC   324
Asp His Asp Val Arg Leu Arg Phe Cys Asp Ile Asp Pro Asp Thr Leu Asn Leu   108

GAC CCG GCG GTG CTG GAG ACG CTG ATC ACC GAC CGC ACC CGG GCG ATC CTG CTC   378
Asp Pro Ala Val Leu Glu Thr Leu Ile Thr Asp Arg Thr Arg Ala Ile Leu Leu   126

GTC CAC TAC GGC GGC AAC CCG GCC GAC ATG GAC CGC ATC ATG GCC CTG GCC CGC   432
Val His Tyr Gly Gly Asn Pro Ala Asp Met Asp Arg Ile Met Ala Leu Ala Arg   144

AAG CGC GGC ATC ATC GTC GTC GAG GAC AGC GCG CAC GCG CTG GGC GCC GTG TAC   486
Lys Arg Gly Ile Ile Val Val Glu Asp Ser Ala His Ala Leu Gly Ala Val Tyr   162

CGG GGG CGG CGG CCG GGG GCA CTG GCG GAC ATC GGC TGC TTC ACT TTC CAC TCC   540
Arg Gly Arg Arg Pro Gly Ala Leu Ala Asp Ile Gly Cys Phe Thr Phe His Ser   180
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ACG AAG AAC ATC ACC ACC CTC GGC GAG GGC GGC ATG ATC ACC CTG TCG CGT GAC 594
Thr Lys Asn Ile Thr Thr Leu Gly Glu Gly Gly Met Ile Thr Leu Ser Arg Asp 198

GAG TGG GCC CAG CGG GTG GGA CGT ATC CGC GAC AAC GAG GCC GAC GGC GTG TAC 648
Glu Trp Ala Gln Arg Val Gly Arg Ile Arg Asp Asn Glu Ala Asp Gly Val Tyr 216

GCG GCG CTG CCG GAC TCC GCG CGG GCG GGT GCT CCG GCG CTG CTG CCG TGG ATG 702
Ala Ala Leu Pro Asp Ser Ala Arg Ala Gly Ala Pro Ala Leu Leu Pro Trp Met 234

AAG TTC GCG GAG GGT GTG TAC GGT CAC CGG GCG GTC GGG GTC CGC GGG GCG GGC 756
Lys Phe Ala Glu Gly Val Tyr Gly His Arg Ala Val Gly Val Arg Gly Ala Gly 252

ACG AAC GCG ACG ATG TCG GAG GCG GCG GCG GCG GTG GGC GTG GTG CAA CTG GCG 810
Thr Asn Ala Thr Met Ser Glu Ala Ala Ala Ala Val Gly Val Val Gln Leu Ala 270

TCG CTG GAG CGG TTC GTG GCC CGG CGC CGG AGC ATC GCG CAG CGG CTG GAC GAG 864
Ser Leu Glu Arg Phe Val Ala Arg Arg Arg Ser Ile Ala Gln Arg Leu Asp Glu 288

GCC GTG GCC TCG GTG GCC GGC ACC CGG CTG CAC CGG GCG GCG GCG GAC AGT CTG 918
Ala Val Ala Ser Val Ala Gly Thr Arg Leu His Arg Ala Ala Ala Asp Ser Leu 306

CAC GCC TAC CAC CTG TAC ACG TTC TTC CTC ACC GGC GGC CGG CAG GTG CGG GAG 972
His Ala Tyr His Leu Tyr Thr Phe Phe Leu Thr Gly Gly Arg Gln Val Arg Glu 324

CGG TTC GTG CGC GCC CTG GAC CGG CTG GGT GTG GAG GTC CAG TTG CGG TAC TTC 1026
Arg Phe Val Arg Ala Leu Asp Arg Leu Gly Val Glu Val Gln Leu Arg Tyr Phe 342

CCG CTC CAT CTG TCG CCC GAG TGG CGG CTG CGC GGC CAC GGG CCG GGC GAG TGT 1080
Pro Leu His Leu Ser Pro Glu Trp Arg Leu Arg Gly His Gly Pro Gly Glu Cys 360

CCG ACG GCC GAA CGG GTC TGG TTC GAG GAG CAC ATG AAC CTG CCG TGC CAT CCC 1134
Pro Thr Ala Glu Arg Val Trp Phe Glu Glu His Met Asn Leu Pro Cys His Pro 378

GGT CTG AGT GAC GGC CAG GTC GAC TAC ATG GTC GAG GCG GTC ACC CGC GCC CTG 1188
Gly Leu Ser Asp Gly Gln Val Asp Tyr Met Val Glu Ala Val Thr Arg Ala Leu 396

CAC GAG GCC CAC GGC ACG GGG ACG CGG GTG GCG GCC GGG CAC CTG 1233
His Glu Ala His Gly Thr Gly Thr Arg Val Ala Ala Gly His Leu 411